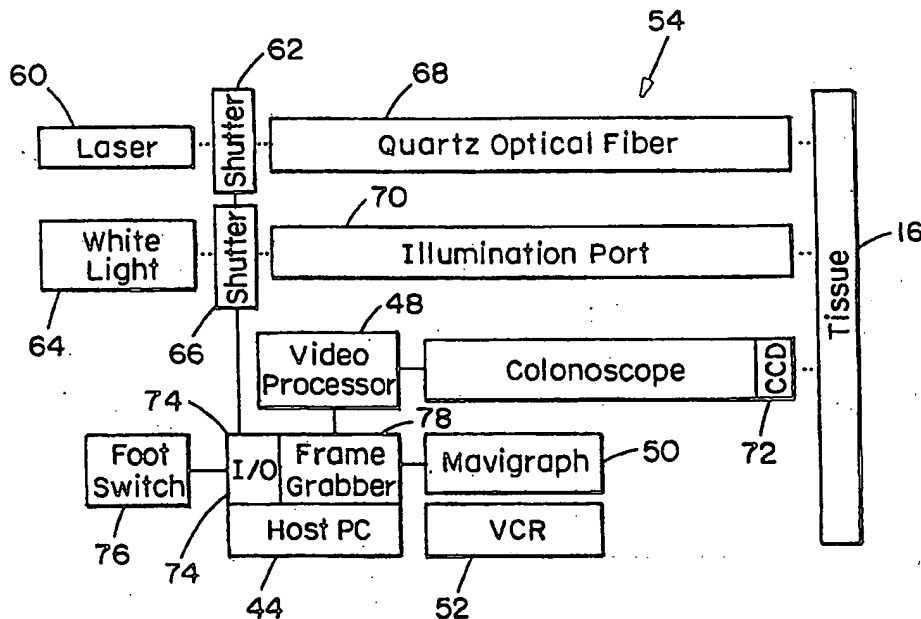




INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | | |
|---|--|--|--|
| (51) International Patent Classification ⁶ : A61B 5/00 | | A1 | (11) International Publication Number: WO 99/37204 |
| | | | (43) International Publication Date: 29 July 1999 (29.07.99) |
| (21) International Application Number: PCT/US99/01723 (22) International Filing Date: 26 January 1999 (26.01.99) (30) Priority Data: 60/072,455 26 January 1998 (26.01.98) US (71) Applicant (for all designated States except US): MASSACHUSETTS INSTITUTE OF TECHNOLOGY [US/US]; 77 Massachusetts Institute, Cambridge, MA 02139 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): WANG, Thomas, D. [US/US]; Apartment 226B, 143 Albany Street, Cambridge, MA 02138 (US). FELD, Michael, S. [US/US]; 56 Hinckley Road, Newton, MA 02163 (US). (74) Agents: HOOVER, Thomas, O. et al.; Hamilton, Brook, Smith & Reynolds, P.C., Two Militia Drive, Lexington, MA 02421 (US). | | (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments. | |

(54) Title: FLUORESCENCE IMAGING ENDOSCOPE



(57) Abstract

The present invention relates to a fluorescence endoscope imaging system (40). The system uses first and second light sources (60, 61) to provide fluorescence and reflectance images of tissue (16) being examined. An imaging device (72) mounted at the distal end of the endoscope (54) is used to collect both images.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | | | |
|----|--------------------------|----|--|----|--|----|--------------------------|
| AL | Albania | ES | Spain | LS | Lesotho | SI | Slovenia |
| AM | Armenia | FI | Finland | LT | Lithuania | SK | Slovakia |
| AT | Austria | FR | France | LU | Luxembourg | SN | Senegal |
| AU | Australia | GA | Gabon | LV | Latvia | SZ | Swaziland |
| AZ | Azerbaijan | GB | United Kingdom | MC | Monaco | TD | Chad |
| BA | Bosnia and Herzegovina | GE | Georgia | MD | Republic of Moldova | TG | Togo |
| BB | Barbados | GH | Ghana | MG | Madagascar | TJ | Tajikistan |
| BE | Belgium | GN | Guinea | MK | The former Yugoslav Republic of Macedonia | TM | Turkmenistan |
| BF | Burkina Faso | GR | Greece | ML | Mali | TR | Turkey |
| BG | Bulgaria | HU | Hungary | MN | Mongolia | TT | Trinidad and Tobago |
| BJ | Benin | IE | Ireland | MR | Mauritania | UA | Ukraine |
| BR | Brazil | IL | Israel | MW | Malawi | UG | Uganda |
| BY | Belarus | IS | Iceland | MX | Mexico | US | United States of America |
| CA | Canada | IT | Italy | NE | Niger | UZ | Uzbekistan |
| CF | Central African Republic | JP | Japan | NL | Netherlands | VN | Viet Nam |
| CG | Congo | KE | Kenya | NO | Norway | YU | Yugoslavia |
| CH | Switzerland | KG | Kyrgyzstan | NZ | New Zealand | ZW | Zimbabwe |
| CI | Côte d'Ivoire | KP | Democratic People's Republic of Korea | PL | Poland | | |
| CM | Cameroon | KR | Republic of Korea | PT | Portugal | | |
| CN | China | KZ | Kazakhstan | RO | Romania | | |
| CU | Cuba | LC | Saint Lucia | RU | Russian Federation | | |
| CZ | Czech Republic | LI | Liechtenstein | SD | Sudan | | |
| DE | Germany | LK | Sri Lanka | SE | Sweden | | |
| DK | Denmark | LR | Liberia | SG | Singapore | | |
| EE | Estonia | | | | | | |

-1-

FLUORESCENCE IMAGING ENDOSCOPE

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application Serial No.60/072,455 filed on January 26, 1998, the entire contents of which is incorporated herein by reference.

5

Background

The following relates to the development of a laser-induced fluorescence imaging endoscope for mapping cancerous or precancerous tissues in hollow organs. In initial clinical studies, on colon polyps, Ultraviolet (UV) light was used at 370 nm to excite visible fluorescence (400-700 nm), the spectral signatures of which enabled differentiating between normal and abnormal tissues. Previously endoscopic imaging has been achieved using an optics module mounted in one of the biopsy ports of a two-port standard (white light) colonoscope. The optics module employs a quartz optical fiber and associated optics to deliver the UV light to the tissue, and a coherent quartz fiber-optic bundle to transmit the resulting fluorescence image to the proximal side of the endoscope, where a filter removes the large background of reflected UV light and the fluorescence image is then captured by a high-gain CID detector array.

15

20

-2-

5 Endoscopically-collected autofluorescence images of
colonic mucosa can be used as a screening tool for
detecting pre-cursors to colorectal cancer (CRC).
Fluorescence has been used to distinguish between normal
mucosa and adenomas. In particular, spectra measured with
10 single point contact probes with the use of several
different excitation wavelengths.

Fluorescence spectra have been obtained through
optical fiber probes with several excitation wavelengths.
An *in vitro* study performed a search over a wide range of
15 excitation wavelengths, and concluded that 370 nm is
optimal for distinguishing between normal mucosa and
adenoma. Both *in vitro* and *in vivo* studies using
adenomatous polyps as a model for dysplasia have shown that
with this wavelength dysplasia has less peak intensity at
20 460 nm and may have increased fluorescence at 680 nm
compared with normal colonic mucosa. Furthermore, the
morphologic basis for these spectral differences have been
studied by fluorescence microscopy. The decreased
fluorescence intensity in polyps was attributed to its
25 raised architecture, increased vasculature, and reduced
collagen in the lamina propria. The red enhancements arise
from increased fluorescence of the crypt cells, which may
be caused by higher levels of porphyrin.

30 Summary of the Invention

The present invention relates to imaging endoscopes
and in particular to a fluorescence imaging colonoscope
using a dual channel electronic endoscope that employs a

-3-

5 charge coupled device (CCD) chip or other solid state
imaging device mounted on its distal tip to collect the
white light image. Of particular significance for the
present invention is that this chip can also collect the
fluorescence image, displaying it on the endoscope's video
10 monitor with much larger signal size than that obtained
using the optics module and intensified CID camera. This
configuration was used to collect fluorescence images of
colonic dysplasia. Video images of two small FAP polyps,
have been taken with the standard white light image and the
15 unprocessed fluorescence image.

The CCD detector, which lacks gain intensification,
detects the weak fluorescence signals, which are six orders
of magnitude smaller in intensity than the diffusely
reflected white light image. In addition, it is surprising
20 that reflected 370 nm excitation light did not completely
flood the CCD, obscuring the fluorescence signal. This
results from the fact that the CCD spectral response falls
off to zero quickly at wavelengths below 400 nm. Thus, the
CCD effectively serves as its own long pass filter. Other
25 imaging devices can be used with a filter to reduce by at
least one half the detected intensity in the ultraviolet
region relative to the detected intensity in the visible
region.

In this particular embodiment, the CCD has a
30 resolution of 270 x 328 pixels and an objective lens of 2.5
mm in diameter. The images are collected in 33 ms in RGB
format. The advantages of this particular embodiment
include that the in vitro fluorescence images exhibit a

-4-

5 signal-to-noise ratio (SNR) of about 34 at clinical working
distances of 20 mm (distance between tip of endoscope and
tissue surface), which is superior to that obtained using
the UV Module/CID detector, which has a SNR of about 18 at
the same distance. The use of the CCD eliminates the need
10 for the optics module and greatly simplifies system design.
In addition, it also avoids problems associated with the
tendency of the UV module to rotate in the biopsy channel.
By using the same detector and optics for white light and
fluorescence images, perfect registration of these two
15 images can be obtained. Parallax between the white light
image of the CCD and the fluorescence image of the optics
module was a significant problem. The CCD in this
particular embodiment contains 88,560 pixels compared to
10,000 fibers for the UV module, resulting in higher total
20 image resolution. The objective lens on the Pentax
colonoscope has better imaging properties than the UV
module. The characteristic width for the line spread
function of the lens of this embodiment is 200 mm compared
to 400 mm for the UV Module. The overall rigidity of the
25 spectral endoscope is not increased significantly with a
single UV illumination fiber.

The diagnostic methods employed can be based on the
overall fluorescence intensity difference between normal
mucosa and dysplasia. Thus, in certain applications it is
30 preferable to collect the fluorescence emission over the
full band between 400-700 nm. However, accurate
measurements can use a point contact device such that
diagnostic information can be obtained by sampling the

-5-

5 fluorescence at a plurality of specific wavelengths such as
460, 600 and 680 nm, for example. For many applications
the preferred range for fluorescence excitation is between
350nm and 420 nm. Endoscopic imaging studies with the
electronic CCD endoscope can include the use of color
10 CCD's, which have the ability provide such information.

Brief Description of the Drawings

Figure 1 is a schematic view of an endoscopic system.

Figure 2 is a schematic view of a solid state imaging
15 device such as a CCD on the distal end of an endoscope.

Figure 3 is a schematic diagram of an endoscopic
system in accordance with the invention.

Figure 4 shows the relative sizes of the illumination
area and fluorescence area.

20 Figure 5 is a schematic diagram of an endoscopic
system.

Figure 6 is a graphical illustration of the average
fluorescence intensity and the measured and predicted
signal to noise(SNR)ratio.

25 Figures 7A and 7B are graphical illustrations of
variation fluorescence intensity between an average of 14
frames and a single frame for normal colonic mucosa and
adenoma, respectively.

Figure 8 is an illustration of the sensitivity of the
30 system as a function of detection threshold values.

Figures 9 and 10 show fluorescence intensity profiles
of tissue with adenoma, and including the moving average
and percent ration values.

-6-

5 Figure 11 is the fluorescence intensity graph showing adenoma, normal and intensity ratio values as a function of pressure exerted on the site with the probe.

Figure 12 is an endoscope system showing the difference in collection geometry between the endoscope and
10 a contact probe.

Figure 13 is a preferred embodiment of an endoscope system in accordance with the invention.

Figure 14A is a preferred embodiment of a fluorescence imaging system in accordance with the invention.

15 Figure 14B illustrates graphically the dependence of radiated power on the input power of a light source emitting in the ultraviolet region of the spectrum.

Figure 14C illustrates a timing diagram for a process acquiring fluorescence and reference images.

20 Figure 15 is a preferred embodiment of a fluorescence imaging system in accordance with the invention.

Detailed Description

The equations describing the number of signal photons, N_s , collected by a given pixel in an endoscope as a
25 function of the separation distance d and the radial distance ρ on the tissue surface, and the corresponding SNR are as follows:

$$N_s(p) = \frac{\eta_i \lambda_{em} g^2 T_f T_i T_o f_p r_L^2 \epsilon_i (\lambda_{em}, \Delta \lambda) \tan \theta_m^2 P_o(\lambda_{ex}) \Delta t}{hc \quad 8(1 - \cos \theta_m) N_f d^2 \left(1 + \left(\frac{\rho}{d} \right)^2 \right)^{3.5}} \quad (1)$$

-7-

5

$$SNR = \frac{N_s}{\sqrt{N_s + \left(\frac{\sigma_e}{G}\right)^2}} \quad (2)$$

The geometry and certain symbols are defined in Figure 1. Note also, the emission wavelength λ_{em} , pixel array size $g \times g$, fiber optic transmission efficiency T_t , the bandwidth of the filtered emission wavelength $\Delta\lambda$, the fraction of the transmitted energy in this wavelength region T_f , the collective efficiency T_o of the system optics including the long pass filter, lens and eyepiece, incident light energy $P_o(\lambda_e)\Delta t$, h is Planck's constant, c is the speed of light, f_p is the packing fraction of the fiber cores ϵ_t is the quantum efficiency of the tissue, and N_s is the total number of resolution elements. The signal to noise ratio (SNR) is a function of electronic noise σ_e and gain G .

Colorectal cancer constitutes a major national health care problem. The incidence and mortality for carcinomas of the colon and rectum are second only to those of lung in the United States. This suggests that the current screening methods are inadequate for controlling the spread of colon cancer, and that little advancement in detection has occurred in a long time. The five year survival rate for all patients diagnosed is between 35-49%. Colorectal cancer is relatively unresponsive to radiation and chemotherapy, hence surgical resection with wide margins is

-8-

5 the only reliable method of preventing its growth. These tumors spread by direct extension into adjacent structures and by metastasis through the lymphatics and blood vessels. The most common sites of metastatic spread in order are regional lymph nodes, liver, lungs, and bones.

10 The pathophysiology of this disease begins in the epithelial layer of colonic mucosa as dysplastic changes in the crypts cells. This tissue can be accessed by colonoscope, and if the pre-malignant lesions are detected at an early stage, they can be removed for biopsy. Most
15 carcinomas of the colon and rectum are believed to arise from visible precursor lesions called adenomatous polyps. These benign masses evolve from a monoclonal expansion of epithelial cells which develop irregularities in the size and shape of the nuclei and cytoplasm, a condition known as dysplasia. These lesions can be detected on colonoscopy by
20 their raised architecture. The medically accepted adenoma-carcinoma sequence suggests that colorectal carcinoma arises from adenomatous tissue that undergoes malignant transformation, which is believed to occur through a multi-
25 step process in which genetic alterations accumulate. The presence of a precursor stage in the development of CRC provides a window of opportunity for early detection and removal of these lesions to prevent future progression into carcinoma.

30 The prevalent screening method of colonoscopy relies on the observation of large structural changes in the colonic mucosa in order to locate adenomatous tissue for biopsy. However, this procedure is relatively insensitive

-9-

5 to adenomatous tissue which is flat. Patients diagnosed
with ulcerative colitis (UC), for example, have a high risk
of developing carcinoma from non-polypoid regions of
tissue. Moreover recent studies have concluded that some
forms of adenocarcinoma arise from small superficial
10 adenomas. Because of this risk, frequent screening by
colonoscopy must be performed with multiple biopsies
throughout the colon. However, the likelihood of sampling
error and missed diagnoses in these patients renders this
form of surveillance highly unsatisfactory. Also, the
15 examination of a tissue biopsy is time consuming and
costly. Moreover, considerable intra- and inter-observer
variation occurs in the identification of dysplasia. A
patient who is diagnosed as positive for dysplasia often
must return to the clinic for further screening and
20 possibly for surgical resection of the colon. Thus, the
current state of endoscopic surveillance with histologic
interpretation is an imperfect science and is in need of
improved methodologies with greater sensitivity and
specificity and less intra- and inter-observer variation.

25 The method of fluorescence endoscopic imaging offers
features which can overcome the present screening
limitations with white light endoscopy. This method is
sensitive to the biochemical constituents and
microarchitecture below the tissue surface. Furthermore,
30 combined with endoscopes, fluorescence images can scan wide
areas, and can resolve tissue surfaces on the sub-
millimeter scale. If sufficient information is present on
the fluorescence, computers can be used to determine the

-10-

5 presence and location of diseased regions in real-time.
Autofluorescence has demonstrated the ability to
distinguish between normal and neoplastic human tissue.
The first studies showed that single point fluorescence
spectra can be used to detect tumors *in vitro* from several
10 types of tissue,. Later, *in vivo* studies were performed
for detecting neoplasia in bladder, brain, colon, cervix,
esophagus, lung, oral mucosa, and skin. In addition,
fluorescence has been used to distinguish normal tissue
from diseased with the use of exogeneous agent such as
15 hematoporphyrin derivative (HpD).

 The full length from the rectum to the cecum is
typically 1.5 m. Histologically, the mucosa is the layer
in contact with the lumen, and has a thickness of about 400
 μm . The epithelium is the most superficial layer and
20 consists of absorptive columnar cells and intermittent
mucin-producing goblet cells, which function to reabsorb
water and to lubricate. These cells undergo continuous
turnover, and are replaced by rapidly dividing stem cells
at the base of the crypts, where the first signs of
25 dysplasia can be observed. The surrounding lamina propria
contains blood and lymphatic capillaries which supports the
secretory, absorptive and other highly active functions of
the mucosa. It consists of loose connective tissue, in
particular collagen, along with numerous inflammatory cells
30 which protect the intestinal wall from invasion by
microbes.

 The muscularis mucosa is composed of several layers of
smooth muscle fibers which contract to expel secretions

-11-

5 from the glandular crypts, prevents clogging, and enhances
absorption by maintaining contact between epithelium and
luminal contents. The submucosa contains the larger blood
vessels, lymphatics, and nerves, and are surrounded by
dense collagenous connective tissue which keeps the mucosa
10 attached to the muscularis propria. The muscularis propria
contains an inner circular and outer longitudinal muscle
layer, which are involved in the involuntary peristaltic
contractions of the colon for propagating the flow of fecal
matter. The outer serosal layer consists of connective
15 tissue which contain the major blood vessels and nerves.

Adenomatous polyps are raised protrusions of mucosa
which contain immature, poorly differentiated epithelial
cells with irregularity in size and shape of the nuclei.
These lesions are benign but they have the potential to
20 transform into colorectal carcinoma. The different
morphological types include tubular, villous, and
tubulovillous adenomas. Although all forms are raised,
each type can either contain a stalk, which is called
pedunculated, or can be hemispheric, which is known as
25 sessile. The malignant potential of polyps are greatest
with the villous form and least with the tubular. Also,
the probability of carcinoma developing increases with the
size of the polyp. There is about a 1% chance of finding
invasive tumor in a polyp less than 1 cm in diameter, 10%
30 for polyps between 1 and 2 cm, and 45% for polyps larger
than 2 cm. The sub-cellular changes associated with these
polyps are frequently histologically identical to the
dysplasia found in ulcerative colitis.

-12-

5 Results of molecular biology studies suggest that the
steps involved in the malignant transformation of adenoma
into carcinoma involves the mutational activation of an
oncogene coupled with the sequential loss of several tumor
suppressor genes. Also, it was found that several genes
10 must incur mutations before malignant tumors arise.
Several specific genetic alterations have been identified
during the process of tumorigenesis. Activational
mutations have been found in the ras oncogene of 50% of
colorectal carcinoma. Furthermore, allelic deletions were
15 identified in portions of chromosomes 5, 17, and 18, which
may involve loss of tumor-suppressor genes.

 Patients with the presence of over 100 neoplastic
polyps in their colon are diagnosed with the condition
called familial adenomatous polyposis. These people have a
20 genetic predisposition for developing numerous polyps in
their colon by adulthood. Most patients have between 500
and 2500 polyps, and on average, there are about 100
polyps. FAP is a rare disease, and accounts for only about
1% of the incidence of CRC in the Western world. Foci of
25 dysplasia usually become malignant, and FAP patients must
have their colons removed at a young age. The probability
for the onset of colon cancer for someone with this
condition is 10% at 10 years of age, 50% at 20 years, and
100% at age 30. Histologically, most of the polyps are
30 tubular adenomas with a high probability of malignant
transformation, and the dysplasia associated with FAP
polyps is identical to that found in sporadic polyps. An
autosomal dominant genetic defect is responsible for the

-13-

5 development of this disease.

A second form of CRC that is associated with familial predisposition is hereditary nonpolyposis colorectal cancer (HNPCC). HNPCC is defined as patients with at least three relatives in two generations having CRC, and with at least
10 one relative being diagnosed at less than 50 years old. This form is much more common than FAP, and accounts for up to 13% of the incidences of CRC in the Western world. HNPCC patients do not have numerous adenomatous polyps, and it is very difficult to distinguish it from sporadic cases.
15 Genetic linkage has been found between this disease and anonymous microsatellite markers on chromosome 2.

In ulcerative colitis, the mucosa undergoes cytological changes resulting in the formation of dysplasia without the presence of polyp formation. These changes are
20 believed to be associated with repeated episodes of chronic inflammation and repair of the colonic epithelium, and flat, ulcerated tumors with poorly defined margins are common. Patients who have had UC for over 8 years are recommended to have periodic colonoscopy with random
25 biopsies taken. This screening process is not effective because less than 0.1 percent of the total mucosal surface area is sampled. However, it is important to note that only 1% of new incidences of CRC arise from UC cases.

UC is an inflammatory disorder of the colorectal
30 mucosa of unknown cause. Patients with UC are at increased risk for developing dysplasia or cancer. Recognition of this increased risk has resulted in colonoscopic surveillance strategies starting at 7-10 years after the

-14-

5 initial presentation of symptoms. Colonic surveillance
strategies include direct macroscopic visualization of
colonic mucosa and access to mucosal biopsies for
microscopic assessment of dysplasia. Although the
pathological classification of dysplasia was standardized
10 in 1983, differences and inconsistencies remain regarding
the interpretation of dysplasia.

Dysplasia is typically focal. Despite the practice of
taking 12-20 mucosal biopsies during surveillance
colonoscopy, less than 1% of the colonic surface is
15 sampled, so the likelihood of missing small foci of
dysplasia is high. Thus, cancers can develop in patients
without any previous or concurrent dysplasia. Although
performing prophylactic colectomy on all patients after the
first decade of disease would be the most definitive
20 solution to the cancer problem in UC, patients with minimal
or mild symptoms of the disease are understandably
reluctant to take this radical approach. Colonoscopic
surveillance with histologic interpretation remains an
imperfect science in need of improved methodologies with
25 greater sensitivity and specificity.

Furthermore, studies have suggested that flat
dysplasia may be the origin of sporadic colon cancer which
does not arise via the adenoma-carcinoma sequence. The
morphological characteristics of adenomas that proliferate
30 superficially in flat nonpolypoid mucosa have been observed
endoscopically as small plaquelike lesions with vague
redness or discoloration. In a comprehensive study, 33
such lesions were described as slightly elevated with a

-15-

5 reddish surface and a central depression. Foci of cancer or severe atypia were found in 25% of lesions of diameter up to 4 mm, 40% of lesions measuring between 5 and 8 mm, and 80% of lesions with diameter between 9 and 10 mm.

10 There are several methods in practice for the early screening for CRC, but each is limited in its effectiveness. The goal of screening is to detect localized superficial masses in asymptomatic individuals.

15 A sigmoidoscopy involves the clinician viewing the patient's rectum and sigmoid colon with either a rigid or flexible imaging device. This form of screening is based on the finding that 60% of CRC occur within the distal 25 cm of the colon. This length is reachable with a rigid sigmoidoscope, and a flexible one can reach up to 60 cm. However, recent statistics have shown that an increasing
20 number of tumors are found beyond the reach of this device. An advantage of this procedure is that it can be performed without the patient undergoing anesthesia or taking a prep. The most extensive method of screening for this disease is a colonoscopy, where the patient is first prepped and
25 sedated. A colonoscope is inserted throughout the full length of the colon, and the mucosal surface is viewed by the physician under white light for polyps and other abnormal masses. This procedure is adequate for identifying raised lesions, but flat region of dysplasia
30 will go undetected.

The fluorescence of tissue occurs through a process in which the electrons of a biological molecule enters an elevated energy state upon absorbing laser light at a given

-16-

5 excitation wavelength λ_{ex} . The excited state is unstable,
and the electrons will return to the ground state. Most of
this energy is lost as heat through molecular collisions,
but a small fraction of excited electrons undergo an
internal conversion and spontaneously radiates light at
10 longer emission wavelengths λ_{em} . The fraction of molecules
which release energy by fluorescence is called the quantum
efficiency of the tissue, denoted as ϵ_t . The fluorescence
intensity depends on the product of the initial population
of the excited state and the tissue quantum efficiency.

15 The spectral lineshape is determined by the
fluorescence emission and absorption by biochemical
molecules which are unique to the composition of tissue.
The electronic levels of the singlet state are split into
vibrational and rotational states, which in large molecules
20 consists of small intervals and may overlap due to
molecular interactions. The electrons may decay to any of
the vibrational-rotational levels of the ground state,
thus, the fluorescence spectra of biomolecules are
typically broad. This lack of structure in the spectra
25 limits the amount of information that can be obtained from
fluorescence. The tissue components which produce
fluorescence are known as fluorophores, and endogenous
chromophores include aromatic amino acids, NADH, FAD, and
porphyrins. The local environment may have a large effect
30 on the fluorescence emission, which may become quenched or
shifted in wavelength. Further details regarding the use
of outafluorescence for imaging tissue can be found in U.S.
Patent Nos. 4,193,142, 5,421,337, 5,452,723, 5,280,788 and

-17-

5 5,345,941, the entire contents of these patents being
incorporated herein by reference.

 A first step taken in evaluating the use of
fluorescence in colon was to determine the existence of
optimal wavelengths to differentiate between normal colon
10 mucosa and adenomatous polyps *in vitro* with single point
measurements on a sub-millimeter scale. For example, the
fluorescence emissions of 4 normal colon and 11 adenomatous
polyps were recorded with a spectrofluorimeter. The
excitation wavelengths used ranged between 250 to 500 nm in
15 10 nm steps, and the results were tabulated in an array
called an excitation-emission matrix (EEM). A ratio was
taken of the average EEM from the normal colon to that of
the adenomatous polyps, and excitation at 330, 370, and 430
nm were found to produce fluorescence spectra which
20 contained the greatest amount of diagnostic information.

 Based on the results of these *in vitro* studies,
clinical trials were conducted to evaluate the ability of
fluorescence to distinguish among normal, adenomatous, and
hyperplastic colon tissue with 370 nm. In this study, a
25 pulsed nitrogen-pumped dye laser delivered 370 nm
excitation through an optical fiber probe with one
excitation and six collection fibers. This probe was
inserted through the biopsy channel of a colonoscope, and
placed in contact with the colonic mucosa during
30 colonoscopy. The probe consisted of six individual 200 μ m
collection fibers arranged in a bundle with one fiber for
excitation. With this device, fluorescence emission was
detected from an area of tissue about 1 mm². The

-18-

5 fluorescence spectra were detected by a spectrograph
coupled to an OMA. The spectra showed a difference at 460
nm where the normal mucosa produced about 6 times greater
fluorescence intensity than adenoma. This difference is
almost twice that found from the *in vitro* studies. Above
10 650 nm, the average of the adenomas were slightly greater
than that of normal.

From 20 patients, the fluorescence intensities at 460
and 680 nm were located on a scatter plot, and a straight
line was drawn to minimize the number of misclassifications
15 when compared to histology. The decision line correctly
classified 31 of 31 adenomas, 3 of 4 hyperplastic polyps,
and 31 of 32 normal colonic tissue specimens. The
sensitivity, specificity and positive predictive value of
the technique for diagnosing adenomas were 100%, 97%, and
20 94% respectively. Because only a small number of
hyperplastic polyps were examined, it was unclear whether
adenoma could be reliably distinguished from hyperplasia
using fluorescence. The observed differences in the
fluorescence may arise from architectural differences
25 between polyps and the normal mucosa rather than from
dysplastic changes.

The next step was to use the data from this study to
provide prospective methods of evaluating the performance
of fluorescence. The data were randomly divided into two
30 equal sets, and the first was used to devise an algorithm
to distinguish the tissue type based on the fluorescence
intensity at 460 nm and at the ratio between intensities at
680 to that at 600 nm. A biopsy of tissue from each point

-19-

5 was classified histologically as adenomatous, hyperplastic, or normal. From the prospective decision criteria, the sensitivity, specificity and positive predictive value of the algorithm for diagnosing adenomas were 90%, 95%, and 90% respectively.

10 Further attempts have been made to use fluorescence to distinguish between normal mucosa, adenomatous polyps and hyperplastic polyps in vivo with 337 nm excitation. Fluorescence spectra were measured from 86 normal colonic sites, 35 hyperplastic polyps, and 49 adenomatous polyps
15 with a single optical fiber. The fluorescence emission displayed peaks at 390 and 460 nm, which was attributed to the collagen in the submucosa. Also, this peak decreased in intensity for normal mucosa, hyperplastic polyps, and adenomas, respectively. The peak intensity of the normal
20 mucosa was found to be slightly less than twice that for adenomas. Using a MVLR analysis, the sensitivity, specificity, positive predictive value, and negative predictive value of fluorescence to distinguish between adenomatous and hyperplastic polyps were 86%, 77%, 86% and
25 77%, respectively. This study concluded that the differences in fluorescence were due to polyp morphology rather than to the fluorophores present in the polyps.

Other excitation wavelengths have been used to study fluorescence in colon. A continuous wave He-Cd laser was
30 used to deliver 325 nm excitation to measure fluorescence spectra from 35 normal mucosa and 35 adenomatous polyps in vitro from a single optical fiber by an OMA. The peak intensity from normal mucosa occurred at 375 nm and that

-20-

5 for adenoma appeared at 450 nm. A multi-variate linear regression (MVLRL) analysis established a set of scores for each data point to determine a diagnostic criterion. Fluorescence spectra from an additional 34 normal, 16 adenomatous, and 16 hyperplastic sites were taken and
10 analyzed prospectively using the established decision criteria. The sensitivity, specificity and positive predictive value of this study to distinguish between normal and adenomatous tissue were found to be 100%, 100%, and 94%, respectively. In addition, 15 of 16 hyperplastic
15 polyps were classified as normal, which is the correct diagnosis because hyperplastic polyps are formed from a thickening of the epithelial layer.

The fluorescence of colon was studied with 410 nm excitation as well. The emission from 450-800 nm was
20 collected with a spectrofluorimeter from 83 biopsy specimens removed during colonoscopy from 45 patients. The intensity of the emission band from 460-530 nm declined from normal to carcinoma to adenomatous mucosa. The peak intensity at 460 nm was about 2.5 times higher for normal
25 mucosa than for adenoma. A stepwise discriminant analysis was performed on the spectra using nine variables. The results compared to histology showed that the process distinguished between normal mucosa and adenoma with a sensitivity and specificity of 88.2% and 95.2%,
30 respectively. The fluorescence emission resulted from the superposition of three bands centered at about 470, 485, and 404 nm.

Thus, there has been extensive research performed

-21-

5 within the last 5 to 10 years to evaluate the use of tissue
autofluorescence to distinguish between adenomatous and
normal mucosa. *In vitro* studies have concluded that 330,
370, and 430 nm are optimal for excitation. Preliminary *in*
10 vivo results indicate that single point fluorescence
detection has a sensitivity, specificity, and positive
predictive value as high as 90%, 95%, and 90% respectively
for such discrimination. Also, the data suggest that the
intrinsic fluorophores can include collagen, NADH, and
15 porphyrin. Hemoglobin is an absorbing chromophore. In
order to make this technique suitable for a clinical
setting, wide area fluorescence detection and processing
must be performed in real time and adapted to conventional
white light endoscopy. These requirements demand the
development of a spectral imaging instrument.

20 Fluorescence microphotographs of unstained frozen
sections were studied to account for the morphological
structures in normal colonic mucosa and adenomatous polyps
which emit fluorescence. The 351 and 364 nm lines from an
argon-ion laser were used for fluorescence excitation, and
25 the emission was collected by a series of barrier filters
with cut-off wavelengths of 420, 475, 515, 570, and 610 nm.
The fluorescence intensity was graded semi-quantitatively
from 1+ to 4+ by a single observer. In normal mucosa,
fluorescence in the spectral band from 420 to 700 from
30 collagen in the lamina propria was graded at 3+ and that in
the submucosa at 4+ in the same emission bandwidth. In the
epithelium, there was faint fluorescence seen from
absorptive cells and none from goblet cells. The H&E

-22-

5 stained section identifies the tissue composition of normal
mucosa. The fluorescence image of the serial unstained
section indicated the fluorescent structures. Several
differences were observed on the fluorescence image of the
serial unstained section indicating the fluorescent
10 structures.

 Several differences were observed on the fluorescence
micrographs of the adenomatous mucosa. First, fewer
collagen fibers were present in the lamina propria,
resulting in less fluorescence intensity from the
15 epithelium. Also, the level of fluorescence seen in the
cytoplasm of crypt cells was recorded at 2+, compared to
+/- seen in normal crypts. Finally, a larger number of
fluorescent granules were present in adenoma. The image of
the H&E stained section include crypt cells from an
20 adenomatous polyp. The fluorescence from the serial
unstained section shows an observable level of
fluorescence, and the number of eosinophils in the lamina
propria is significantly larger than that in normal mucosa.
The submucosa of the adenomatous polyp was graded at 4+,
25 which is the same as that of normal.

 A procedure has been developed to describe the
clinically observed fluorescence in terms of its
microscopic origins. This process combined the intrinsic
fluorescence of each microstructure with its density as a
30 function of tissue depth and the optical turbidity of the
incident and return path. The concentrations of each
fluorophore from clinical fluorescence spectra can then be
extracted. From this procedure, the factors for observing

-23-

5 greater fluorescence intensity from normal mucosa compared
to that from adenomas include: (1) The submucosal
fluorescence is about 10 times brighter than that of the
overlying mucosa. (2) The mucosa attenuates both the
incoming excitation light and the returning fluorescence;
10 if the mucosa is sufficiently thick, the underlying
submucosa cannot contribute, but if it is thin, as in
normal mucosa, attenuation is smaller, resulting in
brighter tissue fluorescence. (3) In addition, the
fluorescence intensity of adenomas is less than that of
15 normal colonic mucosa, perhaps because the dysplastic
crypts tend to displace the collagen in the lamina propria,
which is the dominant fluorophore. (4) Adenomas exhibit
greater attenuation of both the 370 nm excitation light and
the return fluorescence, due to increased hemoglobin-rich
20 microvasculature.

A multi-spectral imaging system has been developed
which collects fluorescence at four different emission
wavelengths simultaneously. In this device, the output of
a fiber optic endoscope is passed through 4 spatially
25 separated interference filters. The 4 images are arranged
onto quadrants of an intensified CCD array by adjustable
segments of a multi-mirror system. The CCD or other
imaging device 40 as seen in Figure 2 can have 30,000 pixel
elements or more. The four wavelengths were selected to
30 optimize the contrast in the fluorescence spectra between
normal and diseased tissue. Fluorescence from human
cadaveric aorta was excited with 337 nm, and emissions from
400, 420, 450, and 480 nm were ratioed to produce a

-24-

5 dimensionless contrast function. This function indicated a
value for atherosclerotic plaque that was four times
greater than that for normal artery, and the results were
displayed using a false-color overlay. This instrument was
also able to distinguish between rat tumor and surrounding
10 muscle from fluorescence spectra. Further details
regarding this system are described in Wang, T.D. et. al.,
"Real-time In Vivo Endoscopic Imaging of Fluorescence from
Human Colonic Adenomas", Proceedings of SPIE 1998, 3259,
the entire contents of which is incorporated herein by
15 reference.

The resolution of this design is limited by the fibers
in the imaging bundle. The use of 4 fluorescence emission
wavelengths provides for greater contrast between normal
and diseased tissue and for flexibility in the development
20 of the disease detection process. However, by separating
the fluorescence emission in parallel, the signal is
reduced by a factor of 4, thus lowering the SNR. Also, the
4 spectral images must be aligned onto the detector at
different angles, which poses a challenge for image
25 registration. Furthermore, image processing algorithms
using multiple images increase the computation time, and it
is not clear that the fluorescence contains independent
information at 4 bands. Finally, the fluorescence images
are detected at the proximal end of the endoscope, which
30 poses difficulty in clinical use for registering the white
light image and in navigating the instrument.

A simpler version of the multi-spectral imaging system
has been developed which collects only 2 emission bands.

-25-

5 This design splits the fluorescence emission with a beam
splitter onto two intensified CCD cameras. A helium
cadmium laser delivers excitation light at 442 nm via the
illumination bundle of a fiberoptic bronchoscope. The
fluorescence emission was filtered in 2 bands, one between
10 480 and 520 nm and the other at wavelengths greater than
630 nm. The two spectral images were aligned, and the
intensities were ratioed point by point for discriminating
normal from diseased tissue, and a color image was formed.
This method eliminates the effects of distance and angle of
15 the illuminating light, as well as tissue reflective
properties. A color camera is attached separately for
observing the white light image. This system was tested
clinically on 53 patients and 41 volunteers, and the
results were compared with conventional white light
20 bronchoscopy at 328 sites. The sensitivity on fluorescence
was 73%, which was significantly greater than that of 48%
found on white light in detecting dysplasia and carcinoma
in situ. The two methods were found to have the same
specificity of 94%.

25 In the clinical system, the white light and
fluorescence images were collected with a dual-channel
electronic colonoscope (Pentax EC-3800TL). This model
contains two biopsy channels with diameters of 3.8 and 2.8
mm, respectively. The outer diameter of the endoscope is
30 12.8 mm, and the working length is 1.7 m. The field of
view of the multi-element objective lens has a divergence
half-angle of 60° with a depth of focus ranging between 5
and 100 mm. The white light illumination is produced by a

-26-

5 300 W short-arc xenon lamp. By using the same detector for both white light and fluorescence imaging, perfect registration can be obtained. This feature is ideal for producing a diagnostic overlay.

10 An illumination probe consisted of a 200 μm core diameter optical fiber with NA = 0.40 coupled to a 3 mm diameter BK7 glass microlens ($F\#=-1$). The illumination probe was inserted into one of the instrument channels, and the tip was placed flush with the distal end of the colonoscope. A mode mixer clamped the excitation fiber at
15 the proximal end to maximize the divergence angle of the light. The probe was attached at the proximal end of the colonoscope by a leur lock to prevent movement. A power of 300 mW was delivered to the tissue. The spectral response of the CCD detector (TI TC210) cuts off at about 400 nm,
20 and is negligible at the excitation wavelengths $\lambda_{\text{ex}} = 351$ and 364 nm, thus eliminating the need for a long pass filter to block specular reflection from the excitation light. The two instrument channels allow for the optical fiber illumination probe and the biopsy forceps to be used
25 at the same time. Figure 1 shows a schematic view of the endoscope 10 with an imaging bundle 20, biopsy view of the endoscope 10 with an imaging bundle 20, biopsy channel 12, lens 18, and illumination ports 14. The distal end of the device is positioned at a distance d from the tissue. One
30 problem associated with such a system is the shadows generated by the illumination system. An important feature of the invention described below is a process to compensate for shadows on the tissue 16 surface.

-27-

5 A footswitch was activated by the user to block the
excitation light when the white light was used for
illumination, and vice versa, using a pair of computer
controlled shutters (Uniblitz, VS14). The integration time
for acquiring each fluorescence image is 33 ms. As shown
10 in Figure 3, the clinical fluorescence imaging system 40
consists a video processor 48, computer 44, monitor 46,
mavigraph 50, and VCR 52, laser 42 and colonoscope 54.

 An electronic colonoscope 54 detects photons at the
distal end with a CCD detector. An important aspect of the
15 present invention is that the spectral response of the
Texas Instrument TC-210 CCD detector dropped sufficiently
fast below 400 nm that no diffuse reflection from the UV
excitation was observed. In fact, virtually no specular
reflection, which is several orders of magnitude higher in
20 intensity than diffuse reflectance and fluorescence, was
observed either. Another aspect which made this system
possible was that the detector has sufficient sensitivity
to detect fluorescence from colonic mucosa without the use
of an intensifier. Because the detector is located at the
25 distal end, the optical transmission efficiency is
determined only by the multi-element objective lens
positioned between the detector and the tissue. Another
significant feature of this embodiment of the invention is
that the same chip detects both the white light and
30 fluorescence image, thus perfect registration occurs on the
pseudo-color overlay. Furthermore, no modifications are
necessary to the colonoscope which can impede the
clinician's ability to perform the procedure.

-28-

5 One limitation of this system is the bandwidth
selectivity and spectral resolution of the chip. The TC
210 is a monochrome detector and collects fluorescence over
the full visible spectrum. It is difficult to employ
bandpass filters in front of the CCD because the light is
10 collected at angles as high as 60°. However, RGB detectors
exist which contain pixels which are sensitive to red,
green, and blue light, and can produce fluorescence images
in 3 frames. However the passbands are determined by the
integrated circuit manufacturer of the imaging circuit.
15 Note that a gating mechanism can also be used, which is
desirable for using pulsed lasers as the excitation source.
Other excitation sources can include CW lasers and broad or
narrow band light sources.

 The block diagram of an electronic imaging system
20 operated by switch 76 is shown in Figure 5. An argon-ion
laser 60 delivers UV light through a shutter 62 into a
quartz optical fiber coupled to a microlens located in one
instrument channel of the colonoscope, while the white
light 64 is delivered through shutter 66 the illumination
25 fibers of port 70. The pair of shutters 62, 55 are
computer-controlled by a digital input/output (I/O) card
74. Both the fluorescence and white light images are
detected by the CCD 72 at the distal end. A frame grabber
78 digitizes the fluorescence and white light images
30 sequentially. A host microcomputer executes the image
processing algorithm and displays the pseudo-color overlay.
A mavigraph is used to convert the white light image with
overlay into a format which can be recorded by the VCR.

-29-

5 The plot in Figure 6 shows the fluorescence intensity
from the average of 14 frames collected with the electronic
imaging system. A row of pixels is shown from normal
colonic mucosa. Also plotted are the measured and the
predicted SNR. The SNR is approximately 30 at the center
10 and it falls to about 10 near the periphery. Thus, the
full field of view satisfies the minimum SNR requirement of
4 for the instrument-noise limited detection for
distinguishing between normal colonic mucosa and adenomas.

 A frame-to-frame variation from average in the
15 fluorescence image intensities can be seen in Figures 7A
and 7B, which show the differences between the values
across a row of pixels in a single frame compared to the
average of 14 frames. The plot in Figure 7A is that for
the normal specimen shown in Figure 6, and the plot in
20 Figure 7B is from a sample of mucosa which contains an
adenoma in the center. The variation about the average is
small compared to the difference in fluorescence intensity
between normal and adenomatous tissue. Thus, the
occurrence of false positives resulting from pixel-to-pixel
25 variation is small.

 A streaking artifact appeared in the fluorescence
images taken with the electronic imaging system. This
artifact arose because the UV excitation light was not
blocked while the CCD rows were being read out
30 electronically, which is performed under normal white light
illumination by a rotating wheel with spatially separated
filter. This artifact can be removed in the processing
software of the image data.

-30-

5 A study was performed to determine the level of UV
light which can be safely delivered onto the colonic
mucosa. White light and fluorescence images were collected
sequentially. Fluorescence images from 30 patients with 14
colonic adenomas and 6 hyperplastic polyps were collected.
10 Finally, the fluorescence images were collected in parallel
with single point EEM spectra. From these studies, the
effectiveness of the realtime implementation of
fluorescence image collection, processing, and display with
movement in the colon were assessed. In addition, sources
15 of artifact present on the colonic mucosa such as mucous,
stool, and prep were evaluated. Also, the anatomy of the
colon makes it desirable to collect images at large
incident angles, and the effectiveness of the moving
average algorithm with these limitations were determined.
20 Finally, the intensities from fluorescence images were
compared to that from the single point optical fiber
probes.

 The excitation source used was a Coherent Innova 328.
This laser is rated for 1 W in the UV, and requires 60 A at
25 208 V of electrical power and 3 gal/min of water. The
excitation light is coupled into an optical fiber device
including lengths of 12.5 and 16.5 m of fiber were required
to deliver the excitation light to the distal end of the
colonoscope.

30 First, the excitation fiber must be incorporated in
the colonoscope. Next, a method is used to rapidly switch
between white light and laser illumination. Finally, a
method of quickly and accurately registering the

-31-

5 fluorescence results with the white light images must be implemented.

The colonoscopy procedures included prep of the patient with 3 oz of Fleet phospho soda mixed with 4 oz of water. There was no measurable fluorescence from the prep mixture using an optical fiber contact probe on colonic
10 mucosa *in vitro* with 370 nm excitation.

Using the electronic endoscope, white light reflectance and fluorescence images were collected sequentially *in vivo* during routine colonoscopy. The white
15 light image can include a vascular pattern of arteries in red, and an outline of a vein in blue. Patches of specular reflection can be seen on the lower half of the images. The fluorescence of normal mucosa appears uniform with an arterial pattern interspersed as reduced fluorescence
20 intensity. This effect is attributed to the absorption of fluorescence emission by hemoglobin. The vein does not appear on the fluorescence image, and there is virtually no specular reflection from the excitation light. The illumination field on fluorescence is slightly smaller than
25 that on white light, as depicted in Figure 4.

An example illustrates the process of image collection, processing and evaluation of adenomatous polyps. A white light endoscopic image taken of a sporadic polyp located in the rectum shows a polyp with visible
30 architectural features about 5 mm in diameter is located in the lower half of the image near the middle. In the raw fluorescence image the adenoma appears as a region of reduced intensity surrounding a brighter central region.

-32-

5 This image was ratioed with its own moving average
image, and multiplied by 100 to produce the percent ratio
image. Thresholds on the processed fluorescence images
taken at 60%, 75%, and 90% were used to determine the
10 contour lines which define regions of mucosa with various
likelihoods of containing dysplasia. The contours were
then filled in pseudocolor to highlight areas of tissue to
be targeted for biopsy. The pseudocolors red, green and
blue designate regions on the white light image which have
high, medium and low probability, respectively. The polyp
15 was found to be adenomatous on histology.

 Overlay regions indicating disease included one
located at the site of the adenoma, and the other two
corresponded to shadows cast by mucosal folds. The shadows
appeared as regions of reduced intensity on the
20 fluorescence image. These effects were minimized by
directing the endoscope normal to the mucosal surface.
Moreover, the overlay regions which resulted from shadows
changed in size and shape as the angle of the endoscope to
the tissue surface varied, while those generated from the
25 adenoma remained fixed in size.

 White light and fluorescence images were collected
from a total of 30 patients undergoing routine colonoscopy,
which included images from 14 adenomas and 5 hyperplastic
polyps. A biopsy was taken of each adenoma and one
30 adjacent normal site. The fluorescence images were
processed by the moving average algorithm, and the
sensitivity of detection was determined as a function of
threshold values ranging from 55% to 90%. The results of

-33-

5 sensitivity are plotted in Figure 8.

Autofluorescence images of colonic mucosa can be collected endoscopically *in vivo* and can be used to identify and localize dysplasia in the form of adenomatous polyps. The SNR of the fluorescence images was typically
10 above 30. The adenomas were correctly identified by the fluorescence algorithm with high sensitivity. As shown in Figure 8, the sensitivity of *in vivo* detection when the images are collected at normal incidence is comparable to that from the *in vitro* studies. At a threshold of 75%, the
15 sensitivity for detection of colonic adenomas was 86%, compared to that of 92% for the *in vitro* experiments. In order to determine the specificity, the true negatives and false positives must be identified. However, true negatives (false positives) correspond to regions of normal
20 mucosa which were found to be normal (diseased) on fluorescence. These results were not obtained because additional biopsies incur additional risk of perforation. Furthermore, the fluorescence from hyperplastic polyps, which are not dysplastic, did not result in regions of
25 disease from the moving average algorithm.

In comparison of image size, the *in vivo* images encompassed regions of mucosa as large as 10 x 10 cm², whereas the specimens of colonic mucosa were only 2 x 2 cm² in the *in vitro* study. In such large fields of view, the
30 colon contains many mucosal folds, and these layers of tissue blocked the excitation light from reaching the posteriorly-located normal mucosa, thus creating shadows. These folds were not present in the *in vitro* studies.

-34-

5 Diagnostic errors on the processed fluorescence image
resulted primarily from these shadows. The fluorescence
method used is based upon the difference in intensity
between normal and dysplastic mucosa. However, shadows
appear as regions of reduced intensity without dysplasia
10 being present. This artifact can be explained by the
fluorescence excitation geometry. The fluorescence
excitation is provided by one fiber located in the biopsy
port for convenience. The center of this instrument
channel is 8.3 mm away from the center of the CCD detector.
15 The white light image, on the other hand, is illuminated by
two fibers whose centers are located only 3.8 mm from the
detector. Thus, the shadows on the white light image are
much less pronounced than those on fluorescence.

The fluorescence technique used a single fluorescence
20 emission band for detection of adenomas. This method
worked well *in vitro* when the colonoscope is placed at
normal incidence to the lesion, and no mucosal folds were
present. However, during the clinical use of the
fluorescence prototype, the view of the endoscope was often
25 limited to the side of the adenoma. Because the colon is
a tube-shaped structure, some adenomas were anatomically
located at sites where it was virtually impossible to
orient the colonoscope at normal incidence to the lesion.
As a result, one side of the lesion may not be surrounded
30 by normal colonic mucosa. Another situation was that the
normal mucosa is far away to produce fluorescence
intensities sufficiently higher than that of the adenoma.

The fluorescence intensities were measured from the

-35-

5 raw images. The normalized intensity values and the
intensity ratios were taken at three sites within the
adenoma (denoted by left, center, and right in Table 3).
The plot in Figure 9 contains fluorescence intensity
profiles through the adenoma, representing the raw
10 fluorescence and percent ratio values, respectively. The
adenoma was approximately 8 mm in diameter. On
fluorescence, the lesion is located between the 11 mm and
the 19 mm markings on the abscissa, which are labeled by
the vertical lines near the x-axis in Figure 9. Most of
15 the adenomas exhibited a single fluorescence intensity
minimum at the center of the lesion; the average ratio
between normal and diseased pixels was 1.8 ± 0.5 at the
center, and 2.0 ± 0.6 and 2.0 ± 0.7 at the left and right
midpoints, respectively. The average intensity ratio at
20 these sites was 2.0 ± 0.6 . The results of this procedure
show that the differences between normal colonic mucosa and
adenomas for *in vivo* fluorescence images are very similar
to that *in vitro*.

Similarly, the fluorescence intensities were measured
25 from the raw images for hyperplastic polyps. The
normalized intensity values and the intensity ratios were
taken at three sites within the polyp (denoted by left,
center, and right in Table 3). The plot in Figure 10 shows
the fluorescence intensity profiles through the
30 hyperplastic polyp, representing the raw fluorescence and
percent ratio values, respectively. The hyperplastic polyp
was approximately 5 mm in diameter. On fluorescence, the
lesion is located between the 17 mm and the 22 mm markings

-36-

5 on the abscissa, which are labeled by the vertical lines
near the x-axis in Figure 10. The hyperplastic polyps
exhibited an approximately uniform fluorescence intensity
across the lesion which was continuous with the normal
colonic mucosa. The average ratio between normal and
10 diseased pixels was 1.1 ± 0.1 at the center, and 1.2 ± 0.1
and 1.1 ± 0.2 at the left and right midpoints,
respectively. The average intensity ratio at these sites
was 1.1 ± 0.2 . Because this average ratio value is not
significantly different from that of normal mucosa, it is
15 not surprising that no region of disease could be
identified by this intensity method.

In the *in vivo* images, the vascular pattern was
clearly displayed on both the white light and fluorescence
images. The vessels were not apparent on the *in vitro*
20 images, perhaps because the blood supply of the living
colon was no longer intact. The hemoglobin in the blood is
a well-known absorber of light, and produces linear
patterns of weak fluorescence intensity. Thus, the
intensities were measured from the raw fluorescence images
25 of blood vessels. As shown in Table 3, the intensity ratio
from the blood vessels is 1.3 ± 0.1 . This value is
significantly less than the average from adenomas, thus
blood vessels will not present as a source of artifact on
the overlay. Furthermore, image processing methods can be
30 used to remove the blood vessels based on their shape. In
Table 3, the intensity ratios for adenomas, hyperplastic
polyps, and blood vessels are summarized for comparison.

Endoscopic images and single point spectra can both

-37-

5 provide valuable information about tissue biochemistry.
Each method has its own advantages and disadvantages. The
endoscope collects images, and provides spatial information
with sub-millimeter resolution. The fluorescence intensity
between normal mucosa and adenomas can be compared from the
10 same image field within a fraction of a mm from each other.
Also, fluorescence images are collected remotely, thus the
pressure on the tissue is uniform throughout the image
field. However, it is more difficult to acquire spectral
information with fluorescence. Because of the larger areas
15 involved, the fluorescence energy may become too weak at
each pixel to maintain sufficient SNR, unless very large
excitation power is used.

On the other hand, single point optical fiber contact
probes collect fluorescence from an area of approximately 1
20 mm in diameter only. With an intensified optical
multi-channel analyzer (OMA), spectra over a wide bandwidth
can be measured with good spectral resolution and high SNR.
However, the probe must be placed at several sites on the
mucosa to sample differences between normal and adenoma.
25 Typically, the normal mucosa sampled is several cm away
from the adenoma, and comparisons of the absolute intensity
can be affected by biological variability over distance.

The degree of contact of the probe on the polyp can
vary during the *in vivo* measurements because the colonic
30 musculature is constantly contracting and expanding. As a
result, movement is created which makes probe placement
difficult. The adenoma is round and slippery, and the
movement of the colonic wall renders complete contact with

-38-

5 the surface of the polyp very difficult. Furthermore, the distal end of the optical fiber probe is not flat, but there is a 17° bevel. Thus, the orientation of the beveled side will affect the degree of contact as well.

10 Results of the colonoscopy procedure showed that it was very difficult to place the probe onto the polyp for the 0.5 seconds required to collect a full EEM. Light escaping at various colors representing the excitation sources was observed on the normal mucosa surrounding the adenoma. This observation suggests that the delivery of
15 excitation energy to the polyp and collection of fluorescence emission was not complete. Probe contact was hindered by the physiological movement of the mucosa, and by the fact that a flat probe was being placed on a slippery, hemispherical surface. Contact is not a problem
20 for spectra collected on normal mucosa because this surface is flat.

Moreover, the ratios between the intensities of normal mucosa and adenomas can be affected by difference in the pressure exerted on each site. An *in vitro* experiment was
25 conducted on a resected specimen of colonic mucosa which contained an adenoma. The fluorescence intensity in the spectral range between 400 and 700 nm was measured as a function of pressure exerted by the probe which was passed through the biopsy channel of a colonoscope. The pressure
30 was measured with a balance. As shown in Figure 11, the fluorescence intensity increases with pressure, and the intensity ratio does not change if equal pressure is exerted on both the normal and adenoma sites. However,

-39-

5 this is usually not the case during the clinical
acquisition of spectra. The normal mucosa is relatively
flat, and measurements can be made with virtually complete
probe contact with a few grams of pressure. On the other
hand, the pressure on the polyp cannot be made the same as
10 that on the normal site because the probe will slip off.
The pressure on the normal site was estimated to be about 5
grams, while that on the adenoma was estimated to be close
to zero. Thus, the difference in pressure exerted on the
normal mucosa and the adenoma may result in the intensity
15 ratio increasing from 2 to 3, as shown in Figure 11.

Furthermore, on the recorded images of the colonoscopy
procedures, the normal mucosa showed an indentation at the
site where the probe was placed during the collection of
spectra. This observation confirmed the estimate that
20 several grams pressure was exerted on normal mucosa during
data collection. On the other hand, the probe was seen to
slide off the polyp when any significant pressure was
exerted, which resulted from the moistness of the surface.
Thus, the pressure exerted on adenomas was significantly
25 less.

Another procedure was conducted *in vitro* to compare
the fluorescence intensity ratio between normal mucosa and
adenoma as measured on imaging and single point. White
light and fluorescence images of a resected specimen of
30 colonic mucosa containing two adenomas were obtained. The
intensities were measured from 7 normal sites immediately
adjacent to the adenomas on both imaging and single point.
The results included the intensities that were normalized

-40-

5 so that the average value is 100 for each system. This
step allows for direct comparisons to be made at each
point, and reveals that the intensities are within about
10% of each other. Furthermore, the normalized intensity
values range from 68 to 155 on imaging and from 63 to 136
10 on single point. Thus, the intensities measured on normal
mucosa depend on the site sampled with both methods, and
can vary by over a factor of 2.

In Table 4, the normalized intensities and the
intensity ratios are determined for the two adenomas on
15 imaging and single point. These values are determined at
the center and the left and right midpoints of the
adenomas. For the left adenoma, the average intensity
ratio was 1.43 on imaging and 1.54 on single point. For
the right adenoma, the average intensity ratio was 1.52 on
20 imaging and 1.72 on single point. These results indicate
there is little difference in the intensity ratios between
imaging and single point *in vitro*.

The fluorescence intensity ratio was calculated from
Monte-Carlo simulations to determine the fluorescence
25 intensity ratio, given the different excitation and
collection geometries of the imaging system and single
point. In Figure 12, a diagram of the collection geometry
for the endoscope 100 and the single point probe 102 is
shown. The endoscope contains a 2.5 mm diameter objective
30 lens 104, and is located in air at a distance 20 from the
surface of the tissue. This geometry corresponds to a
collection angle of 40°. The probe contains a quartz
shield 106 which is in contact with the tissue 16. The

-41-

5 optical fibers are located at a distance of 2 mm from the tissue surface by this shield 106, and collect light at a NA = 0.22, which corresponds to a collection angle of 12.7°. The optical parameters of colonic mucosa for the excitation and emission wavelengths are shown in Table 2.

10 The excitation used in the simulation is an infinitely-thin beam with a divergence angle of 0°. The fluorescence intensity at a point on the tissue from a uniform thick excitation beam can be determined from the fluorescence collected from a superposition of
15 infinitely-thin excitation beams which are incrementally displaced in distance from the point to be measured. However, this result is equivalent to integrating the fluorescence intensity over the field of view. The LSF of the tissue falls off quickly within several mm, thus the
20 simulation integrates over a 2 mm region within the collection angle specified in Table 5. The results of the simulation are shown in terms of the intensity ratio between the light collected at the tissue surface with that of the excitation. In Table 5, the intensity ratio between
25 normal colonic mucosa and adenomas is 3.0 and 2.9 for the endoscope and the probe, respectively. The intensity ratio is similar for the endoscope and the probe, a result which is consistent with the *in vitro* studies. The intensity ratio for the endoscope is slightly higher than that of the
30 probe, which is consistent with the collection angle of the endoscope being smaller. Light from the highly fluorescent submucosa is more likely to reach the detector with a smaller collection angle.

-42-

5 A model was developed to quantify the number of
photons collected by the endoscopic imaging system over the
field of view at normal angle of incidence. This result is
valid for both white light reflectance and fluorescence
images, and can be applied to both the fiber optic imaging
10 bundles and electronic imaging systems. The spatial
distribution of the illumination and emission profiles of
in the center and to fall off towards the periphery of the
image. When combined with the detector noise statistics,
the SNR of the image can also be determined. This analysis
15 showed that distance and optical collection geometry
produces a profile in which the SNR at the periphery was
always lower than that in the center. This parameter is
needed for developing algorithms for identifying tissue
lesions. Also, the collected light intensity was found to
20 decrease with the square of the distance between the distal
end of the endoscope and the tissue. Furthermore, the
light collection by coherent imaging bundles is limited by
the numerical aperture of the optical fiber. This analytic
tool can be used to design the optical parameters of the
25 fluorescence imaging system and to identify the type of
light source required to excited the fluorescence.

The methods developed for endoscopic imaging model
were used to determine the excitation source, optics, and
detectors necessary for building two fluorescence imaging
30 systems. The first design consisted of a fiber optic
colonoscope which detected the fluorescence image at the
proximal end with an intensified CID camera. A 400 nm long
pass filter was used to block the reflected excitation

-43-

5 light, an a quartz optical fiber located external to the
colonoscope was used for image excitation. The second
design was a modification of the first to accommodate the
requirements for clinical use. This system used an
electronic colonoscope with dual instrument channels, and
10 detected fluorescence images at the distal end. The cutoff
in spectral sensitivity of the CCD detector below 400 nm
was used to avoid the reflected excitation light. An
illumination probe with a high NA quartz optical fiber was
coupled to a microlens and inserted into one instrument
15 channel for image excitation. In both systems, the
excitation source was an argon-ion laser which delivered
about 300 mW at $\lambda_{ex} = 351$ and 364 nm, and microcomputer
with a frame grabber was used to acquire, process, and
display the diagnostic images.

20 Autofluorescence images from human colonic adenomas
were collected with the fiber optic system with high SNR in
vitro. For wide area surveillance of the colon wall,
regions of mucosa as large as 100 mm² must be illuminated.
Furthermore, the endoscopic images are collected remotely,
25 and the intensity collected falls with distance d squared.
Previously, fluorescence spectra were collected from
contact probes which illuminated an area of about 1 mm².
The results of this study showed that excitation sources,
optics, and detectors used in this design could collect
30 autofluorescence images with sufficient SNR to distinguish
between normal colonic mucosa and adenomas. In the fiber
optic system, an SNR of over 30 was attained, which
exceeded the minimum SNR requirement of 7.

-44-

5 Fluorescence images were then collected from samples
of resected colonic mucosa *in vitro* to evaluate the
potential use of this technique for wide area surveillance
of dysplasia. Colectomy specimens from three patients with
familial adenomatous polyposis containing polypoid and
10 non-polypoid adenomas were studied. Each raw image was
corrected for differences in distance and instrument light
collection efficiency by normalizing to a spatially
averaged image. Intensity thresholding was then used to
identify diseased regions of mucosa. The sensitivity and
15 specificity for detecting a region of dysplasia depended on
the threshold value selected. With the threshold set to
75% of the average normal intensity, a sensitivity of 90%
and a specificity of 92% were achieved. The average
fluorescence intensity from normal mucosa was found to be
20 greater than that from the adenomas by a factor of $2.2 \pm$
0.6. These results demonstrate the potential of this
technique to direct biopsy site selection.

The results from the *in vitro* studies provided
motivation for conducting an *in vivo* study. The electronic
25 system was used to collect autofluorescence images from
colonic adenomas *in vivo*. In this system, an SNR of
over 30 was attained as well, which exceeded the minimum
SNR requirement of 4. Fluorescence images were collected
from 14 adenomas and 6 hyperplastic polyps from 30 patients
30 undergoing routine colonoscopy. The fluorescence images
were collected in a 33 ms frames, and were processed by
dividing the raw fluorescence image with a moving average
image. The processed images displayed regions of mucosa

-45-

5 with a probability of containing dysplasia in the form of
adenomas, as verified on histology. With the threshold set
to 75% of the average normal intensity, a sensitivity of
86% was achieved for detecting adenomas and a specificity
of 100% was attained for hyperplastics. On average, the
10 ratio between the fluorescence intensity of normal mucosa
to that from adenomas was 2.0 ± 0.6 and to that from
hyperplastic polyps was 1.1 ± 0.2 . The diseased regions on
fluorescence best corresponded to the adenoma on white
light when the colonoscope was at normal incidence. At
15 higher angles there were greater effects from shadows.
These results showed that dysplasia can be identified on
fluorescence images *in vivo*.

In the single point optical fiber contact probe
studies the average intensity ratio between the
20 fluorescence at 460 nm from normal colonic mucosa and
adenomas was found to be about 3, while that in endoscopic
imaging this ratio was measured to be 2.0 ± 0.6 . Direct
comparison of fluorescence imaging and single point
measurements *in vitro* revealed that there was little
25 difference between the intensity ratio measured on imaging
compared to that measured from single point. There are two
possibilities that can account for the difference in
intensity ratio between the two methods. First, the ratio
of 3 measured by the single point method was performed *in*
30 *vivo*. A lower ratio may have resulted *in vitro* because of
the loss of blood flow, which is known to absorb light.

Alternatively, the difference in the ratios may result
from contact and pressure artifacts. Videotapes of the

-46-

5 colonoscopy procedure showed that it was very difficult to
place the probe onto the polyp for the 0.5 seconds required
to collect a full EEM. Light at various color representing
the excitation sources was observed, which indicated that
the delivery of excitation energy to the polyp and
10 collection of fluorescence emission was not complete.
Probe contact was hindered by the physiological movement of
the mucosa, and by the fact that a flat probe was being
placed on a slippery, hemispherical surface. Contact is
not a problem for spectra collected on normal mucosa
15 because this surface is flat. Furthermore, increased
pressure was found to elevate the fluorescence intensity
collected. Higher pressures were exerted on the normal
mucosa compared to that on the polyp. The probe was seen
to slide off the polyp when any significant pressure was
20 exerted. Both differences in contact and pressure *in vivo*
resulted in a higher ratio between normal mucosa and
adenoma. On the other hand, the fluorescence images are
collected remotely, and the pressure and contact parameters
are identical for normal mucosa and adenoma.

25 Finally, the results of the clinical studies
identified future directions to improve the sensitivity and
clinical usefulness of fluorescence endoscopic imaging.
The shadow artifact can be reduced by illuminating the
tissue through the two white light ports. This
30 modification can be accomplished by replacing the glass
fibers with quartz, thus allowing for both white and
excitation light to be transmitted. Furthermore, the
shadow artifact, angle of incidence, and detection yield

-47-

5 can all be improved by collecting multi-spectral images consisting of two or more fluorescence images. Lastly, the concurrent collection of EEM spectra can be used to identify new excitation wavelengths which result in higher intensity contrast ratios.

10 Dysplastic tissue exhibits an increase in red fluorescence which can be detected to improve the sensitivity of disease detection. Thus another embodiment includes the collection of multiple emission wavelengths. One method of collecting multiple fluorescence emission
15 wavelengths is to use an electronic endoscope (e.g. Olympus, Model CF I OOTL) with a CCD detector which is sensitive to the red, green, and blue (RGB) regions of the visible spectrum. Fluorescence images from each RGB frame can be captured and processed, providing more detailed
20 information for use in a diagnostic procedure. Furthermore, the use of spectral lineshape information from images at different wavelengths reduces all geometric distortions. The TI TC244 has a quantum efficiency of 30% at 640 nm and 15% at 480 nm [TI Manual, 1994].
25 Extrapolating from the 370 nm imaging data and the EEM data, a SNR of 10:1 in the red and 50:1 in the blue is anticipated.

Performing the detection on the distal end of the electronic colonoscope has many practical advantages.
30 First, the same detector can be used for both white light and fluorescence imaging. A single detector not only results in perfect registration of the two images, but avoids the need to interchange of cameras, which can be

-48-

5 cumbersome. Second, fewer optical elements results in a
transmission efficiency of fluorescence photons which is
significantly higher than that of a fiber optic imaging
bundle. Third, the packing geometry of CCD pixels allow
for minimal loss of surface area of detection, unlike fiber
10 optic imaging bundles which have a hexagonal packing array.

While there are advantages to detecting the
fluorescence image with a distally located CCD, a fiber
optic imaging bundle with proximal detection has advantages
as well. The spectral bands of the distal CCD is limited
15 to the RGB response of the distal detector, while the
fluorescence collected by a fiber optic imaging bundle
could be filtered into an unlimited number of spectral
images. Also, detection of the fluorescence image at the
proximal can allow for detection with a gated intensifier.
20 This device enables use of pulsed lasers.

The EEM study provides valuable guidance about new
imaging strategies. The results indicate that excitation
near 410nm is useful. The contrast between normal and
adenoma tissues provided by the blue fluorescence is
25 greatly enhanced compared to that obtained with our current
excitation wavelength (10:1 versus 2:1). In addition, the
red fluorescence is quite pronounced for adenoma.
Extrapolation of the conclusions of the morphological model
developed using $\lambda_{ex} = 365$ nm to this new excitation
30 wavelength suggests that the blue fluorescence contains
information about both crowding of the crypts and mucosal
thickness, and that the red fluorescence contains
information about crypt cell dysplasia. Hence, collecting

-49-

5 images at red and blue emission wavelengths should provide both high contrast diagnostic images and significant new histological information. In addition, the ratio image can be used to normalize out shadow effects. The next phase of the imaging studies will use 410 nm excitation. A krypton
10 ion laser (Coherent Innova Model 302) will provide 500 mW of power at the two lines 407 and 413 nm. This level of power is adequate to achieve large fluorescence signals in both red and blue bands. This laser will be installed at the BWH Laser Laboratory along with the existing 365 nm
15 argon ion laser.

In addition, multiple excitation wavelengths can be employed. One approach would be to use excitation from the 407 and 413 nm lines of a krypton ion laser to excite the red fluorescence and to retain the 365 and 351 nm lines
20 from argon ion laser to excite the blue fluorescence. Two hardware configurations include (1) a fiber endoscope with a switchable filter wheel between the scope and camera, and (2) a dual-chip endoscope. Such a system has been developed, for example, by American Hospital, Inc., for
25 stereo viewing during endoscopy. One can modify one of the windows on the chip with a spectral cut-off mechanism. The timing of the red-sensitive imaging channel can be synchronized with the excitation light.

The diffuse reflectance image at 407-413 nm can be
30 explored to obtain information about the tissue hemoglobin content. This image can be obtained by installing a filter with the appropriate bandwidth on the rotating wheel in front of the white light source. The approach is to ratio

-50-

5 this reflectance image with the fluorescence images in the red and blue frames. In order to develop the required algorithms, and to decide how to optimize the spectral information collected, an extensive contact probe study with 410 nm excitation can be performed.

10 The shadow artifact obtained using the broadband intensity algorithm with 365 nm excitation can be greatly reduced by use of an improved excitation geometry. Currently, excitation light is delivered through a single quartz fiber located in the biopsy channel located 8.3 mm
15 from the CCD detector. The use of a single illumination beam located a large distance from the CCD chip tends to enhance shadows. In contrast, in the conventional white light images produced by this colonoscope, shadows are minimized by use of two closely spaced white light
20 illumination beams symmetrically positioned on opposite sides of the CCD chip. By replacing the illumination fibers with quartz fibers, the UV light can be delivered through the two white light illumination ports, which are located only 3.8 mm from the CCD detector. Implementing
25 this requires modifying the video processor to enable alternate coupling of white light and laser excitation into the illumination fibers.

 Other spectral endoscope improvements can include: (I) regulating the excitation light intensity on the tissue
30 surface via feedback control. This provides constant illumination, regardless of viewing distance, and is also important for patient safety; (ii) minimizing the streaking effect of the fluorescence excitation on the white light

-51-

5 endoscopic imaging display by timing the fluorescence
excitation to occur during the "blank" periods of the
filter wheel used in the endoscope white-light source.
Feedback control of the excitation light can be
accomplished by measurement of the average intensity on the
10 fluorescence image. The intensity of this average value
will be used to modulate the open period on the shutter or
filter wheel. The streaking effect can be completely
removed by implementing the identical filter wheel for
blocking the excitation light that is used for producing
15 the RGB illumination on the white light mode.

As described above, a large argon-ion laser was used
as a near-LTV excitation source for the imaging studies.
Although adequate for these studies, this light source is
expensive and bulky and operates at only a few discrete
20 wavelengths. Such a laser system with its special
electrical and water cooling requirements cannot be easily
moved, preventing use at multiple sites. Alternative
excitation sources can be considered which include a pulsed
laser and a white-light source with filters, both of which
25 are compact and transportable.

For applications in which near-UV excitation is
appropriate, a pulsed ND:YAG laser is used because it can
provide third harmonic radiation at 355 nm with sufficient
average power for spectral imaging. In both the *in vitro*
30 and *in vivo* studies, good SNR was obtained with 300 mW of
laser power, which corresponds to 10 mJ of energy per
pulse. Therefore, a frequency tripled ND:YAG laser with a
5 - 10 ns pulse duration operating at 30 Hz with an average

-52-

5 power of 300 mW at 355 nm will be adequate. Using a CCD
camera gated at about 10 ns, this short excitation pulse
enables simultaneous acquisition of white light and
fluorescence images. Within this short temporal gate the
white light background is negligible, obviating the need to
10 chop the white light illumination. There are no special
power or water requirements for lasers of this type and a
fluorescence endoscope system with such a laser will be
easily transportable.

A mercury lamp can also be used as an excitation
15 source. Such a source is compact and lightweight and can
provide a bright, narrowband illumination at a number of
excitation wavelengths. Employing this light source
simplifies system design and reduce cost, enabling less
expensive units to be produced for use at multiple sites.
20 The key issue is whether enough light in the desired
wavelength range can be coupled into the illumination
fiber(s). A commercial white light source with a 150 W
xenon lamp is capable of delivering as much as 80 mW of
white light at the distal end. Utilizing a 50 run
25 excitation bandwidth, about 20 mW of light can be used to
induce tissue fluorescence.

At selected wavelengths, mercury lamps have 5 to 10
times higher output powers than that of xenon. This
indicates that with a 500 W mercury lamp having a
30 relatively small filament, at least 300 mW of useful
excitation light should be available at the distal end of
the illumination fibery should be sufficient for collection
of good quality fluorescence images from colonic tissue.

-53-

5 In addition, to further enhance SNR, either the total area of illumination can be reduced or imaging elements can be binned together. A lamp and power supply can be selected for this application with the proper brightness, stability and minimum electrical interference.

10 Currently, the image processing scheme is based on ratioing the raw image to a spatially-averaged image, and applying a threshold criterion for classifying a region of tissue as normal or diseased. The averaging window and detection threshold values are pre-flexed, regardless of
15 the polyp size, viewing angle and distance. These predetermined values limit the range of polyp sizes which can be accurately measured. Improved image processing and thresholding methods will employ variable window sizes for spatial averaging and variable thresholds. Information
20 from the raw digitized image about the diameter of the largest lesion in the image will be used to determine these parameters. This change in the window size as a function of the lesion in the image field will maximize the intensity ratio and optimize the performance of the
25 fluorescence method.

 Image analysis methods for extracting information from multivariate images can also be explored. A multivariate image is a collection of congruent images of the same object measured with different variables, such as reflected
30 wavelengths, or fluorescence or Raman band intensities. Many methods are available for analyzing multivariate images, and they can be adapted to image analysis. In general, three steps will be followed, image processing,

-54-

5 object segmentation, and contrast measurement. The images
will first be processed based on the selected operation,
such as moving-window average, intensity difference or
ratio. The processed image will then be segmented based on
both frequency and intensity information. This can be done
10 either through thresholding, quick/slow descent, or region
growth. These methods can be coupled to the concomitant
identification and display of a lesion(s) based on a
probabilistic scheme.

When techniques for collecting multiple spectral
15 images are developed and a database of such images are
built, more advanced image analysis methods, such as
principal component analysis and regression analysis can be
used. Principal component analysis does not assume a known
(a priori) distribution, but instead employs a set of
20 calibrated data to extract information about structures
exhibiting pre-malignant changes. The regression technique
is based on the principle of building up a mathematical
relationship between two groups of variables, i.e., between
a number of independent variables and one or more dependent
25 variables. As an example, a logistic regression to
correlate spectral intensities in the images with
histopatwogy of dysplastic lesions.

The development of the fluorescence imaging endoscope
has demonstrated the potential to perform wide-area
30 surveillance colonoscopy using fluorescence. The
fluorescence image can be analyzed in real time and can
provide the endoscopist with an instant interpretation of
the probability of dysplasia determined using a

-55-

5 previously-validated algorithm. In addition, the ability
to guide biopsy can be used with the present invention. In
patients with FAP, fluorescence imaging can be used to
direct mucosal biopsies to areas that are endoscopically
normal-appearing (non-polypoid) but, based on their
10 spectral characteristics, can have an increased likelihood
of being dysplastic. Histopathological assessment of
mucosal biopsies will be correlated with spectral data to
validate for detection of "flat" dysplasia.

The following method can be followed for determining
15 the capability of the fluorescence imaging system for
directing biopsy. The entire surface of the colon wall,
both at colonoscopy and using resected samples at
colectomy, is systematically imaged, and isolated areas
which are diagnosed as dysplastic selected for directed
20 biopsy. Random areas diagnosed as benign can also be
sampled, and the spectral diagnosis confirmed by
histological analysis. Again, the effects of complications
such as inflammation can be investigated. Once an imaging
algorithm has been validated, it can be adapted to the
25 detection of dysplasia in patients with UC. As in the case
of the contact probe studies, diagnostic algorithms for UC
must be capable of evaluating patients with various degrees
of background inflammation. The same patient groups
studied with contact probe EEMs will be studied with
30 fluorescence imaging. An important potential benefit of
wide area fluorescence surveillance is that one or more of
the otherwise random biopsies obtained during conventional
surveillance colonoscopy may be directed by the results of

-56-

5 fluorescence imaging. Those biopsies can be separated
from the remainder of the random biopsies to assess whether
fluorescence imaging can increase the yield of dysplasia
detection over random sampling.

10 The development of the rapid EEM and spectral imaging
systems represent two very important advances in
instrumentation. The two systems are complementary. The
imaging system views wide areas of mucosa in real time, and
the EEM system provides complete spectral characterization
of a given site of colonic mucosa. The two instruments can
15 be used simultaneously, where appropriate. The EEM probe
is placed through the second channel of a two channel
colonoscope. Thus, each system can be used to verify the
other. Also incorporated herein is the publication
attached hereto and entitled "Real-Time in vivo endoscope
20 imaging of fluorescence from human colonid adenomas".

The following table compares the signal size expected for white light imaging, fluorescence imaging observed with the endoscope CCD, and fluorescence imaging using the optics module with the intensified CID camera. The parameters listed below are taken from either the manufacturer's specification or from experimental measurements.

| <u>Imaging Device</u> <u>Module</u> | <u>Definition</u> | <u>Pentax(white light)</u> | <u>Pentax(fluorescence)</u> | <u>UV</u> |
|--|-------------------|----------------------------|-----------------------------|-----------|
| λ_{ex} (nm) | ex wavelength | - | 356 | 356 |
| λ_{em} (nm) | em wavelength | - | 460 | 460 |
| $\Delta\lambda$ (nm) | em bandwidth | 400-700 | 400-700 | 400-700 |
| P_o (mW) | power | 1 | 300 | 300 |
| Δt (s) | integration time | 0.011 | 0.011 | 0.033 |
| d (mm) | distance | 20 | 20 | 20 |
| Diameter (mm) | area illum | 70 | 70 | 28 |
| θ_m (degrees) | max angle | 60 | 60 | 35 |
| N_f (pixels/fibers) | number | 88560 | 88560 | 10000 |
| r_L lens (mm) | radius | 1.25 | 1.25 | 0.3 |
| ϵ_t | tissue efficiency | 1 | 5.00E-05 | 5.00E-05 |
| f_o | packing fraction | 1 | 1 | 0.6 |
| T_f | % trans filter | 1 | 1 | 0.8 |
| T_i | % trans imaging | 1 | 1 | 0.9 |
| T_o | % trans optics | 1 | 1 | 0.9 |
| η_c | photocathode eff. | 0.2 | 0.2 | 0.1 |
| g | group factor | 1 | 1 | 1 |
| N_s | signal photons | 1.7x10 ⁵ | 2500 | 338 |
| σ_e | electronic noise | 55 | 55 | 50 |
| G | gain | 1 | 1 | 10,000 |
| SNR | signal/noise | 407 | 34 | 18 |

TABLE 1

-58-

TABLE 2

| | normal | | adenoma | |
|------------------------|----------------------|----------------------|----------------------|----------------------|
| λ (nm) | $\lambda_{ex} = 370$ | $\lambda_{em} = 460$ | $\lambda_{ex} = 370$ | $\lambda_{em} = 460$ |
| μ_a | 0.9 | 0.45 | 2.1 | 1.1 |
| μ_s | 15.0 | 9.5 | 8.5 | 5.9 |
| g | 0.9 | 0.9 | 0.9 | 0.9 |
| mucosal thickness (um) | 450 | 450 | 1000 | 1000 |
| quantum eff(mucosa) | 0.1 | 0.1 | 0.8 | 0.8 |
| quantum eff(submucosa) | 0.1 | 0.1 | 0.8 | 0.8 |

TABLE 3

| | Adenoma | Hyperplastic | Blood Vessels |
|--|-------------|--------------|---------------|
| <i>Normalized Intensity</i> | | | |
| Normal | 100.00±27.5 | 100.0±18.1 | 100.00±16.1 |
| Left | 54.0±16.3 | 87.9±16.3 | |
| Center | 59.9±16.7 | 95.9±16.7 | 75.3±5.1 |
| Right | 54.4±17.7 | 98.1±17.7 | |
| <i>Intensity Ratio (normal/lesion)</i> | | | |
| Average | 2.0±0.6 | 1.1±0.2 | 1.3±0.1 |
| Left | 2.0±0.6 | 1.2±0.1 | |
| Center | 1.8±0.5 | 1.1±0.1 | |
| Right | 2.0±0.7 | 1.1±0.2 | |

TABLE 4

| | Imaging | | Single Point | |
|----------------------|----------------------|-------|----------------------|-------|
| | Normalized Intensity | Ratio | Normalized Intensity | Ratio |
| Left Adenoma | | | | |
| Left | 65 | 1.54 | 58 | 1.72 |
| Center | 74 | 1.35 | 74 | 1.35 |
| Right | 71 | 1.41 | 65 | 1.54 |
| Avg | 70 | 1.43 | 66 | 1.54 |
| Right Adenoma | | | | |
| Left | 61 | 1.64 | 57 | 1.75 |
| Center | 81 | 1.23 | 67 | 1.49 |
| Right | 59 | 1.69 | 52 | 1.92 |
| Avg | 67 | 1.52 | 59 | 1.72 |

TABLE 5

| | endoscope | | probe | |
|------------------|-----------|---------|---------|---------|
| | normal | adenoma | normal | adenoma |
| d (mm) | 20 | 20 | 2 | 2 |
| θ_m (deg) | 4 | 4 | 12.7 | 12.7 |
| n_0 | 1.0 | 1.0 | 1.4 | 1.4 |
| n_1 | 1.4 | 1.4 | 1.4 | 1.4 |
| 370 | 2.8E-02 | 2.8E-02 | 4.2E-03 | 6.2E-04 |
| 460 | 8.8E-05 | 2.9E-05 | 2.8E-03 | 9.7E-04 |
| Ratio | 3.0 | | 2.9 | |

SUBSTITUTE SHEET (RULE 26)

-59-

5 Figure 13 presents a schematic outline of the system
which has been demonstrated in clinical practice in a
format which will allow comparison with the improved
systems to be described below. The embodiment shown uses
an ultraviolet laser source 200, switched by a shutter 202
10 and focused with a lens 204 into a fused silica fiber probe
206 inserted into a biopsy channel of an endoscope 208 to
deliver it to a tissue site 210 so that it can illuminate
the tissue over an area 212. The UV illumination thus
comes from an aperture 214 which is different from the
15 endoscope's own illumination ports 216. In the dual-
channel Pentax endoscopes used in the clinic this procedure
leaves one biopsy channel 218 free.

The endoscope camera 220 obtains its white light
illumination through its own fiberoptic illuminator 222
20 from a broadband Xenon arc lamp 224 and collection optics
226. A non-standard shutter 228 under computer 230 control
232 is attached to allow the white light illumination to be
turned off while fluorescence images are being taken. The
fluorescence image signal 234 is processed by the
25 endoscope's video processor 236 to produce a standard video
signal 238 which is digitized by a framegrabber in computer
230. The processed image signal 240 with its information
on the state of the observed tissue is sent to monitor 242.
The entire diagnostic procedure is initiated by a foot
30 switch 244 attached to the computer by a cable 246.

Figure 14A shows an design for the fluorescence
imaging system which eliminates the tendency of the
previous system to identify shadows in the image as regions

-60-

5 of dysplasia. The improved design uses a 100W mercury (Hg) arc lamp light source 302, dichroic mirrors 304 and 306, wavelength filters 308 and 310 and rotating shutters 312 and 314 to provide precisely-timed, tissue-illumination pulses in two separate wavelength bands. The first
10 wavelength band is centered on the near-ultraviolet (365 nm) mercury resonance line and is used to obtain the UV autofluorescence image. The second wavelength band is at the red end of the visible spectrum and is used to obtain a simultaneous or near-simultaneous, reflectance image for
15 the purpose of identifying shadows and the extent of the UV illumination field.

A reflectance (non-fluorescing) image taken with an endoscope camera system measures the brightness of the tissue surface 316 in its field of view. To the extent that
20 the tissue surface is a Lambertian (non-specular) reflector (generally the case) this image indicates the distance of the tissue from a single illumination source (or a weighted distance from multiple sources). If these illumination sources are not in the direct line-of-sight from the camera
25 to the tissue source there will be shadows. A reflectance image can thus be used to measure both the UV illumination 318 at the tissue surface and the presence of shadows in the fluorescence image as long as the UV illumination and the visible illumination emanate from the same aperture 320
30 with the same angular divergence. Note that this condition can be satisfied either by a two-color illumination fiber 322 passed through a biopsy channel of an endoscope 324 or by the two-color illumination being passed through the

-61-

5 illumination bundle 326 of the endoscope. A shutter 328
switches off the normal white-light illumination of the
endoscope while the two diagnostic images are being
obtained. The closing of shutter 328 under computer
control 329 occurs at the same time as the opening of
10 shutter 330 by control line 331. This action enables the
two-color light to reach the fiber 322 and thus the tissue
316.

The algorithm for using the visible reference image
along with the fluorescence image is as follows. The video
15 signals 332 from the CCD camera at the distal tip of the
endoscope 324 are converted by the video processor 334 to a
standard NTSC color video signal 336 and sent to a video
framegrabber in computer 338. The two images are first
corrected for the gamma factor applied to the video signal
20 by the video processor to insure that the digitized images
acquired by the framegrabber in the computer are linear
measurements of the tissue surface brightness. This is
accomplished in real time by the framegrabber input look-up
table. The two images are then normalized to their peaks,
25 which will generally be a region of non-dysplastic tissue
in the visual field. This normalizes the two illumination
fields. On a pixel-by-pixel basis the fluorescence image
value is then divided by the visible reference image value.
If the ratio falls below a predetermined threshold
30 (typically one-half to one-third) then that pixel in the
image represents a region of reduced fluorescence which is
indicative of dysplasia. This pixel can then be set to a
false color state in an output video signal 340 sent to a

-62-

5 monitor 342 to indicate to the clinician the probability
of dysplasia. A prior threshold requirement on both images
insures that the ratio obtained is significant and
eliminates false color output in regions of shadow or low
illumination at the edges of the video field. This entire
10 operation occurs for every depression of the footswitch 344
which is connected to the computer through cable 346.

In the improved design both the UV-excitation light
pulse and the visible-reference light pulse are delivered
to the tissue through the same optical fiber 322 inserted
15 through a biopsy channel of the endoscope. The condition
that the two illumination sources have the same angular
distribution is assured by the design of the light
collection apparatus shown in Figure 14A. A single Hg arc
lamp 302 is used as the source of both wavelengths. A
20 dichroic mirror 304 reflects the UV portion of the spectrum
and transmits the visible portion. Filters in each path
further refine the bandwidth of the two beams. The UV
filter 308 must reject visible light to a high degree since
the efficiency of the 460 nm tissue fluorescence is only
25 about 0.1%. The filter 310 in the red path is less
critical but the chosen center wavelength should avoid
hemoglobin absorption bands to provide the best reference
image. Note that the design of the beam splitting optics
and beam combining optics have an even number of
30 reflections in both the UV and visible arms. This assures
that any angular deviations of the output beams due to
motion of the lamp track each other. It also makes the
directions of the output beams invariant under translations

-63-

5 and small rotations of the beam splitting and recombining optics as a whole.

In the improved source design the current through the Hg lamp 302 is boosted at appropriate moments to increase the lamp output power for the UV exposure. This allows a
10 larger area of tissue to be scanned for dysplasia in a single image. The data in Figure 14B show that the UV output power from a 100 W Hg lamp is a linear function of its input power to at least a factor of 3 over its nominal rated power. Since the lamp discharge maintains a constant
15 voltage drop across the arc regardless of current, the lamp output power is essentially proportional to current. At least 50% power to the lamp must always be maintained, however, to keep the mercury in the vapor phase. The lamp power supply 348 in the improved fluorescence system
20 utilizes a DC current section to maintain the idle current and a computer-controlled 350, pulsed current section which can rapidly switch in multiple constant-current sources to vary the output power of the lamp as required by the imaging system. If the idling power is kept below the rated
25 power and the current pulses are kept to a sufficiently small duty factor, then the pulsed UV output can be sustained continuously.

The rotating shutter in the UV path 312 and in the red path 314 of the light source are designed to provide pulsed
30 illumination light according to the timing diagram in Figure 14C. This diagram shows how this fluorescence imaging system is used with a monochrome camera, which uses a xenon arc lamp 352 for illumination and a rotating blue-

-64-

5 green-red filter wheel 354 to synthesize a color image from
3 monochrome images. In this type of system a pulse of blue
light first illuminates the tissue for about 6 milliseconds
and the resulting tissue reflectance image is digitized for
the next 6 milliseconds. The illumination must be turned
10 off during the readout period because the monochrome camera
used continues to collect photo current in the pixels as
they are line shifted to the readout electronics.
Illumination during the readout causes a smearing artifact
in the image. The UV illumination is switched in during the
15 normal blue exposure period and the red light illumination
is switched in during the red exposure period. The green
exposure period is not generally used but could be used to
obtain an additional reference image or an additional UV
fluorescence image. The shutters are timed to the video
20 acquisition system using an LM1881 Video Sync Separator
circuit to develop an even/odd frame synchronization pulse
356 from the standard composite video output signal. Phase-
locked-loops (PLL) 358 and 360 synchronize the phase of the
chopper wheels to this signal by varying a voltage to their
25 DC driving motors 362 and 364. This signal is also used to
synchronize the current pulser 348. In the schematic of
Figure 14A the chopper wheels are shown in collimated
portions of the beam. In practice, these chopper wheels are
placed at an internal focal point in the two arms of the
30 optics train (not illustrated) to provide for fast rise and
fall times for the light pulse.

Note that the dual-wavelength illumination method can
also be used with standard, color-CCD camera endoscopes. In

-65-

5 this case the UV light illumination and red light reference
illumination are present simultaneously. The UV-induced
fluorescence (primarily at 460 nm) is then detected by the
blue-responsive pixels in the CCD camera and the reference
reflectance image is detected by the red-responsive pixels.
10 Note that the visible blue light must still be removed from
the diagnostic illumination so as not to decrease the
contrast of the fluorescence image. The slight amount of
red tissue fluorescence seen in dysplastic tissue due to
the UV excitation is much smaller than the level of direct
15 red illumination. The slight increase also acts to reduce
the fluorescence/reference ratio which properly increases
(slightly) the measured probability of dysplasia.

Figure 15 shows a preferred embodiment of the
fluorescence imaging system in which dual wavelength
20 illumination capability as well as white-light illumination
capability is built into the video endoscope system itself
400. This requires the illumination bundle 402 of the
endoscope 404 to be transmissive at UV wavelengths which is
not usually the case with current commercial systems. Such
25 a design fulfills the design requirement that the UV-
excitation and visible-reference illumination emanate from
the same aperture or apertures 406. Such a system would
also be easier for an operator to use since the
illumination fiber would not have to be threaded down
30 through a biopsy channel 408 and those channels would be
free for their standard uses. The tissue surface 410 would
be illuminated over a larger area 412 with fewer shadows
since dual illumination ports 406 are standard. The video

-66-

5 signal 414 would be processed by the endoscope system 416,
formatted into a standard signal 416 and processed by the
computer 420. The output video signal with the false color
overlay 422 would be sent to a monitor 424 for the
10 clinician to see in real time. The diagnostic illumination
would be initiated by a footswitch 426 connected by a cable
428 to the computer 420 with the light pulses controlled by
signal lines 430 and 432 to the shutters in the light
source.

15 While this invention has been particularly shown and
described with references to preferred embodiments thereof,
it will be understood by those skilled in the art that
various changes in form and details may be made therein
without departing from the spirit and scope of the
invention as defined by the appended claims.

20

-67-

5

Claims

1. A method of fluorescence imaging comprising:
providing an endoscope having an optical guide
that is optically coupled a first light source and a
second light source, the endoscope having an image
sensor at a distal end;
collecting a reflectance image with the image
sensor and generating a reference;
collecting a fluorescence image with the image
sensor; and
processing the fluorescence image with the
reference to provide a processed fluorescence image.
2. The method of Claim 1 further comprising providing an
image sensor having a filter to remove ultraviolet
light from light returning from tissue.
3. The method of Claim 1 wherein the image sensor has a
sensitivity in the ultraviolet region that is less
than half of the sensitivity of the sensor in a
visible region.
4. The method of Claim 1 wherein the reference corrects
intensity for shadows in the fluorescence image.
5. The method of Claim 1 wherein the fluorescence image
is non-intensified.
6. The method of Claim 1 wherein the first light source

-68-

- 5 is a broadband source.
7. The method of Claim 1 wherein the second light source generates a wavelength in the range of 350nm to 420 nm.
- 10 8. The method of Claim 1 further comprising compensating for shadows on a tissue surface to be imaged.
9. The method of Claim 1 wherein the first and second light source are optically coupled to the same optical
15 guide.
10. The method of Claim 1 further comprising providing a shutter coupling each light source to an optical
20 fiber.
11. The method of Claim 1 further comprising imaging dysplasia on a tissue surface.
12. A fluorescence imaging system comprising:
25 an endoscope;
a light source coupling to an optical guide extending through the endoscope; and
an imaging sensor at a distal end of the endoscope that detects a fluorescence image and
30 reflectance image of tissue.
13. The imaging system of Claim 12 wherein the light source comprises a first broadband light source and a

-69-

- 5 second narrow band light source.
14. The imaging system of Claim 13 wherein the narrow band
light source emits light having a wavelength in the
range of 350nm to 420nm.
- 10 15. The imaging system of Claim 12 further comprising a
fiber optic device optically coupled to the light
source.
- 15 16. The imaging system of Claim 12 further comprising a
processor that processes the reflectance image and the
fluorescence image and generates compensated
fluorescence image.
- 20 17. The imaging system of Claim 12 further comprising a
shutter positioned along an optical path between the
light source and an optical fiber extending through
the endoscope.
- 25 18. The imaging system of Claim 12 wherein the image
sensor has reduced sensitivity in an ultraviolet
spectral region relative to sensitivity in a visible
spectral region.
- 30 19. The imaging system of Claim 12 wherein the image
sensor comprising a filter reducing image sensor
sensitivity below 400nm.

-70-

- 5 20. The imaging system of Claim 18 wherein the sensor sensitivity in the ultraviolet spectral region is less than one half of the sensitivity in the visible region.

1/11

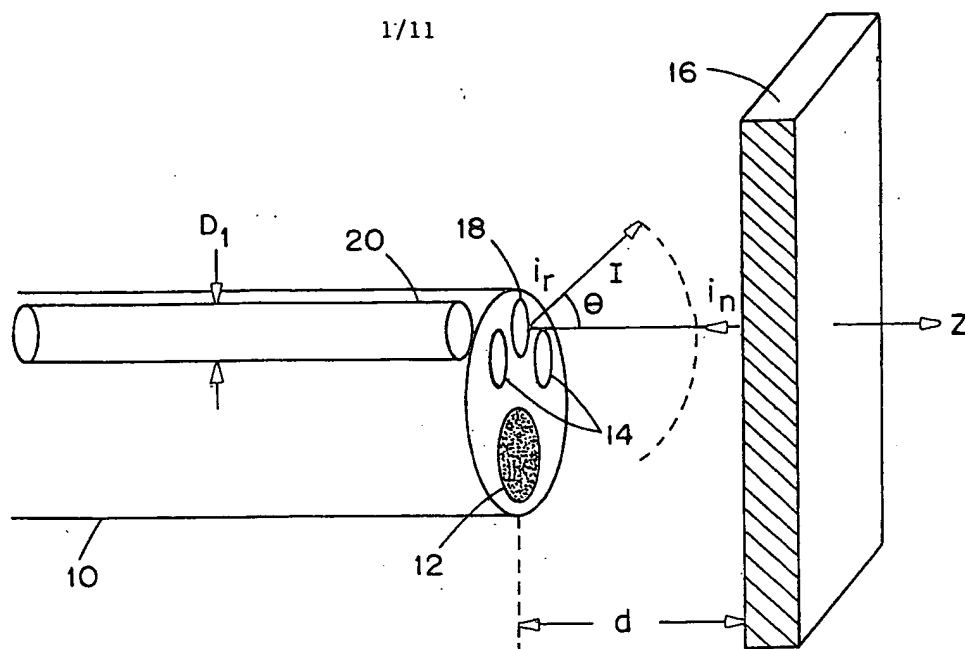


FIG. 1

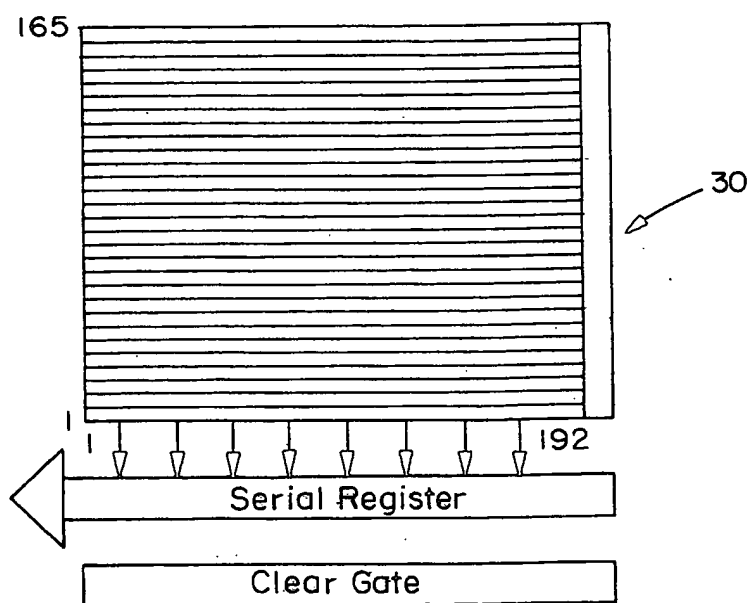


FIG. 2

SUBSTITUTE SHEET (RULE 26)

2/11

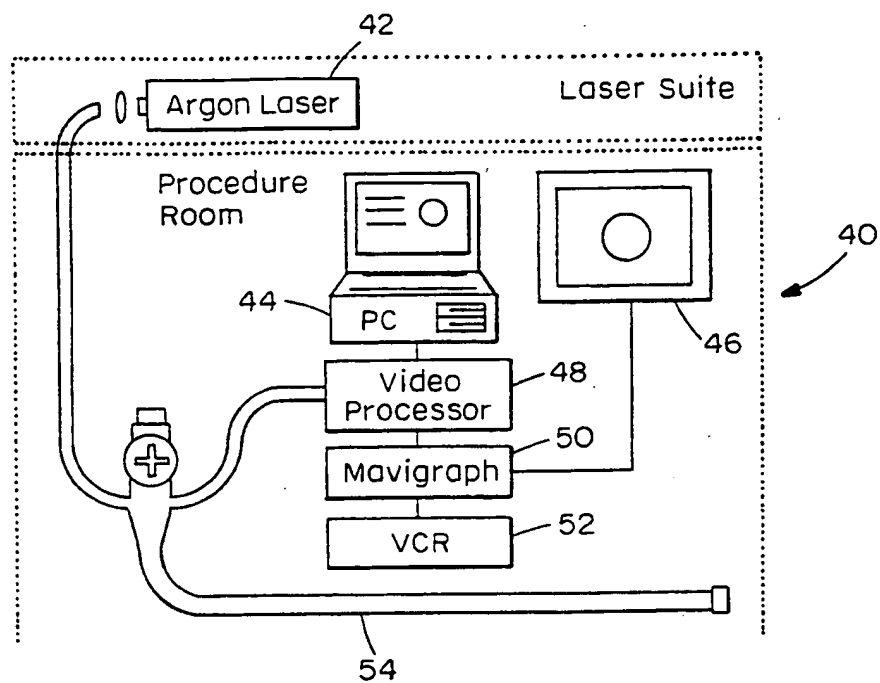


FIG. 3

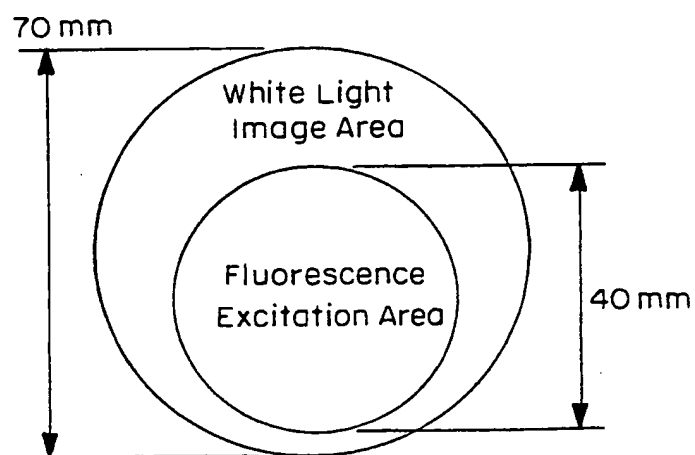


FIG. 4

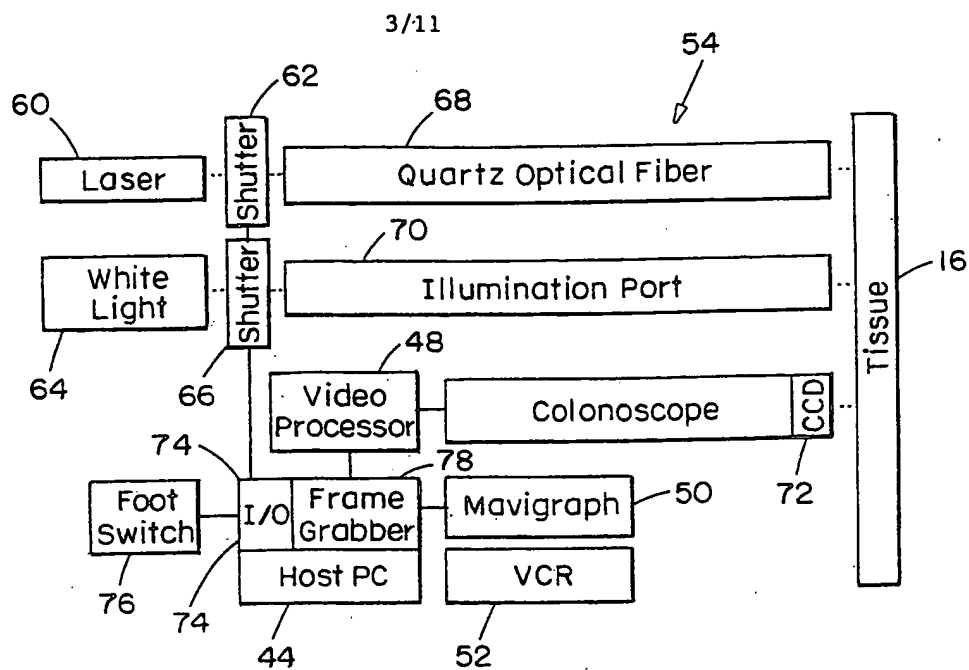


FIG. 5

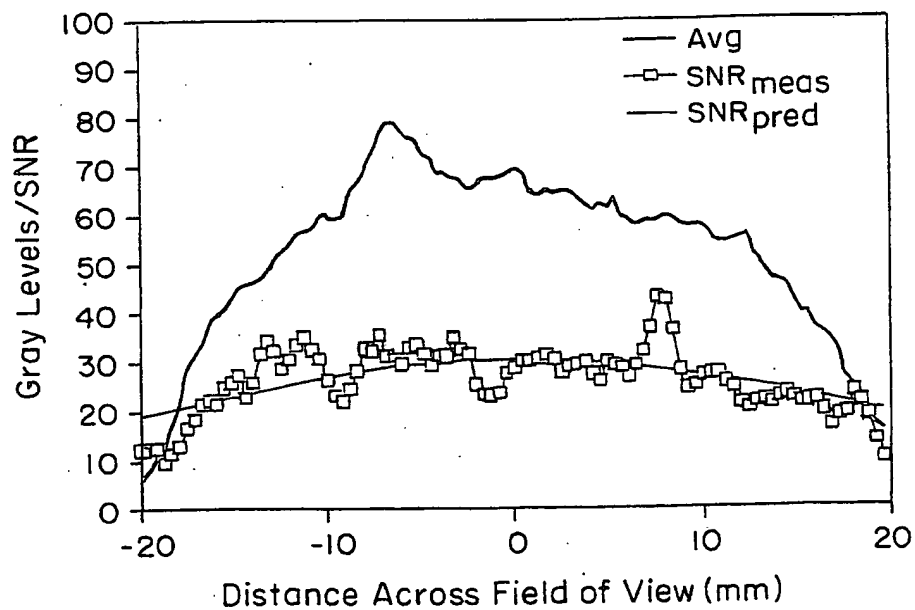
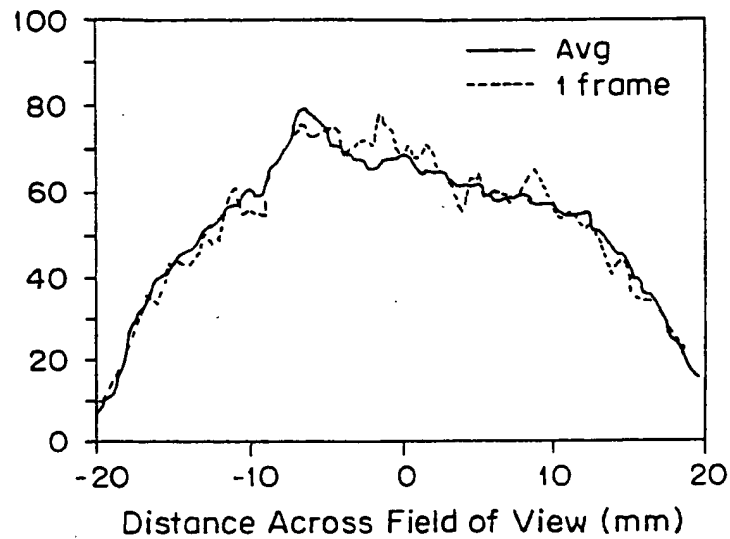
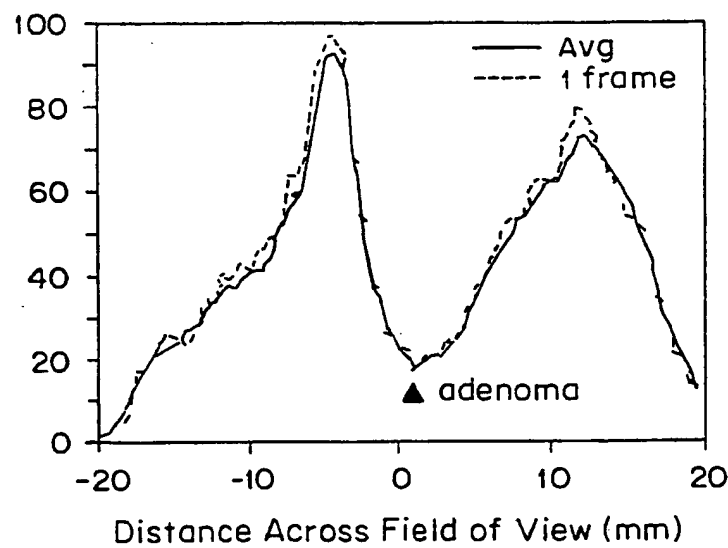


FIG. 6

4/11

**FIG. 7A****FIG. 7B**

5/11

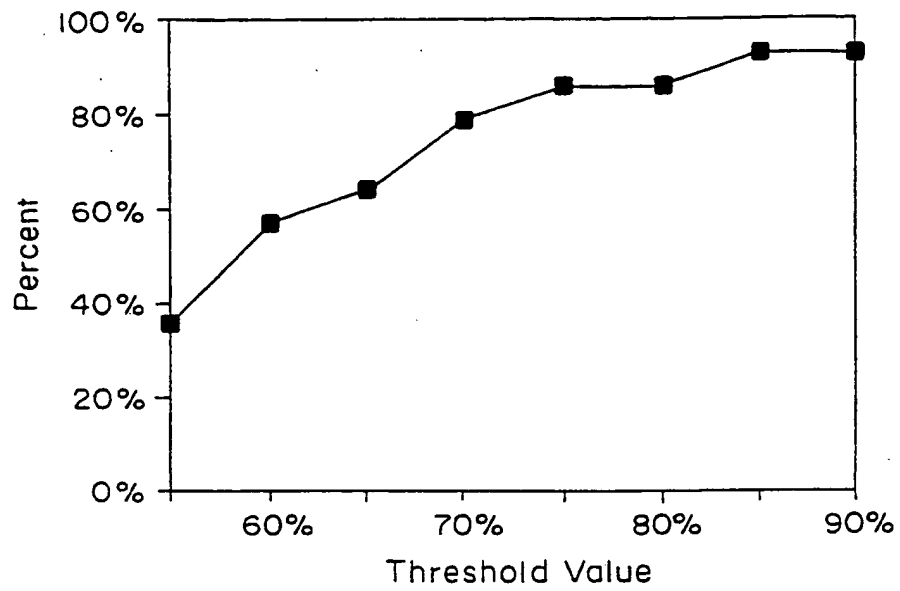


FIG. 8

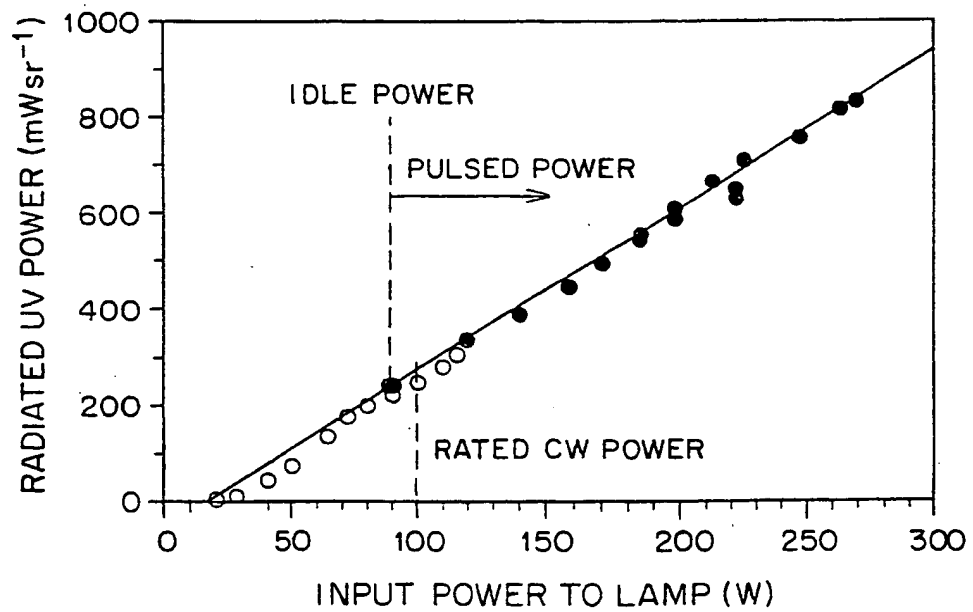


FIG. 14B

6/11

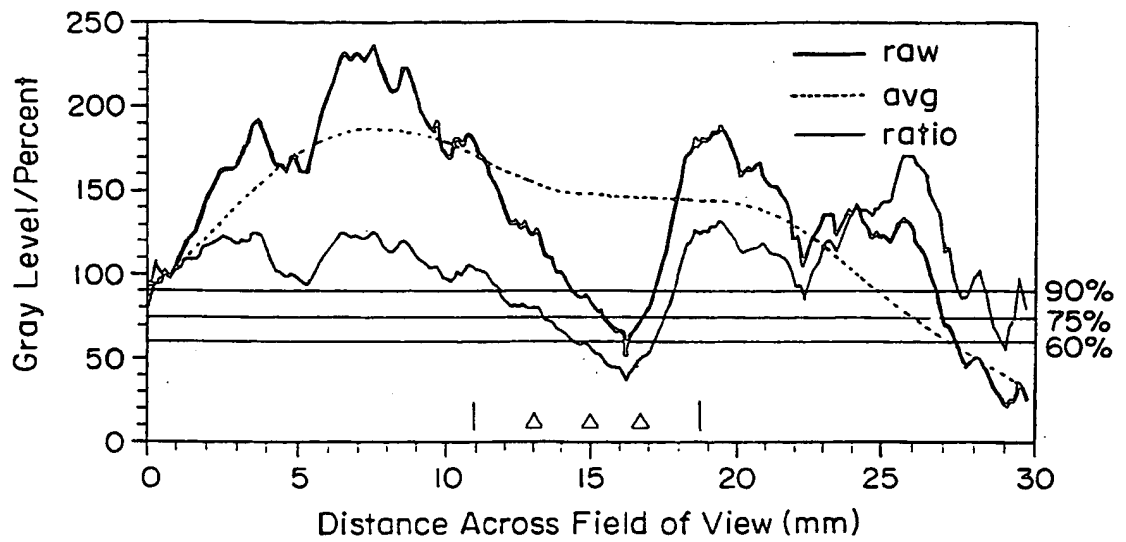


FIG. 9

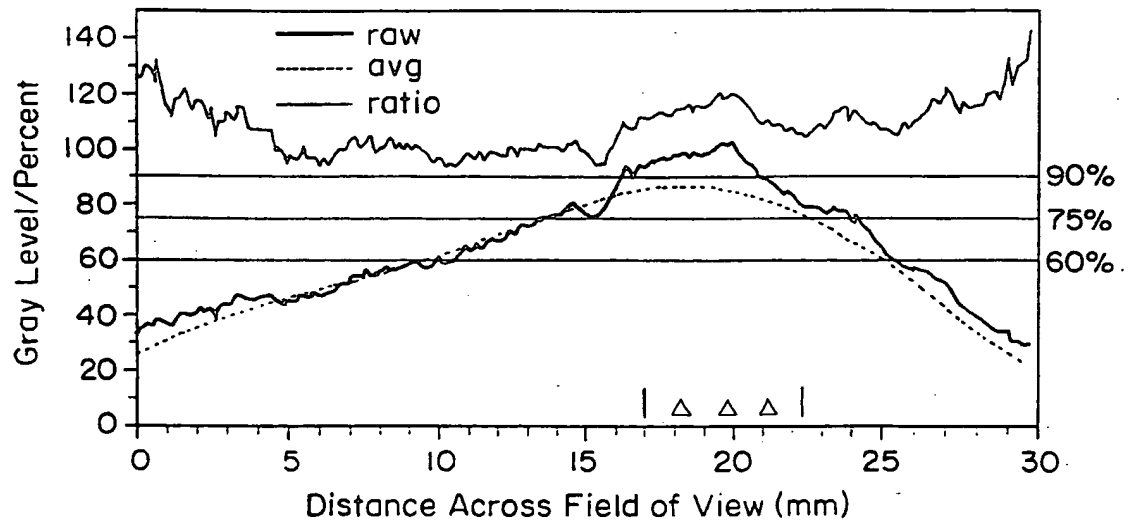


FIG. 10

7/11

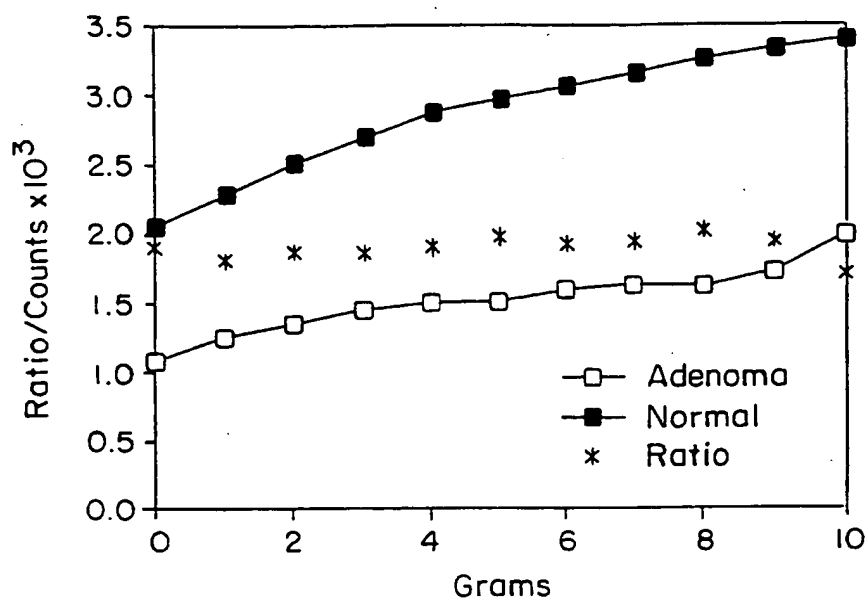


FIG. 11

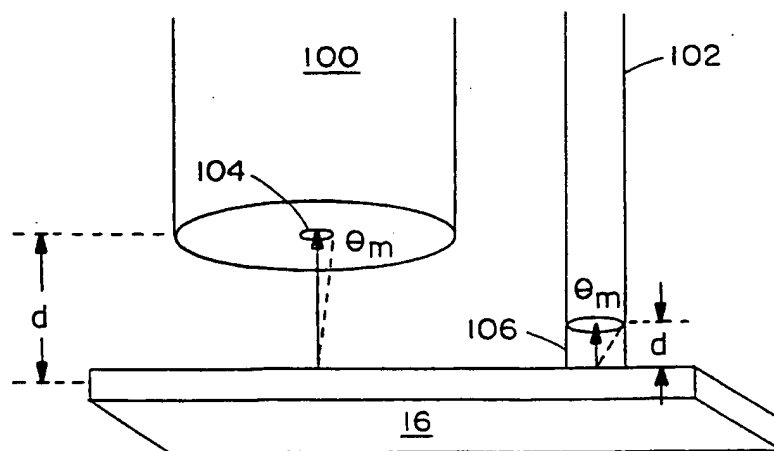


FIG. 12

8/11

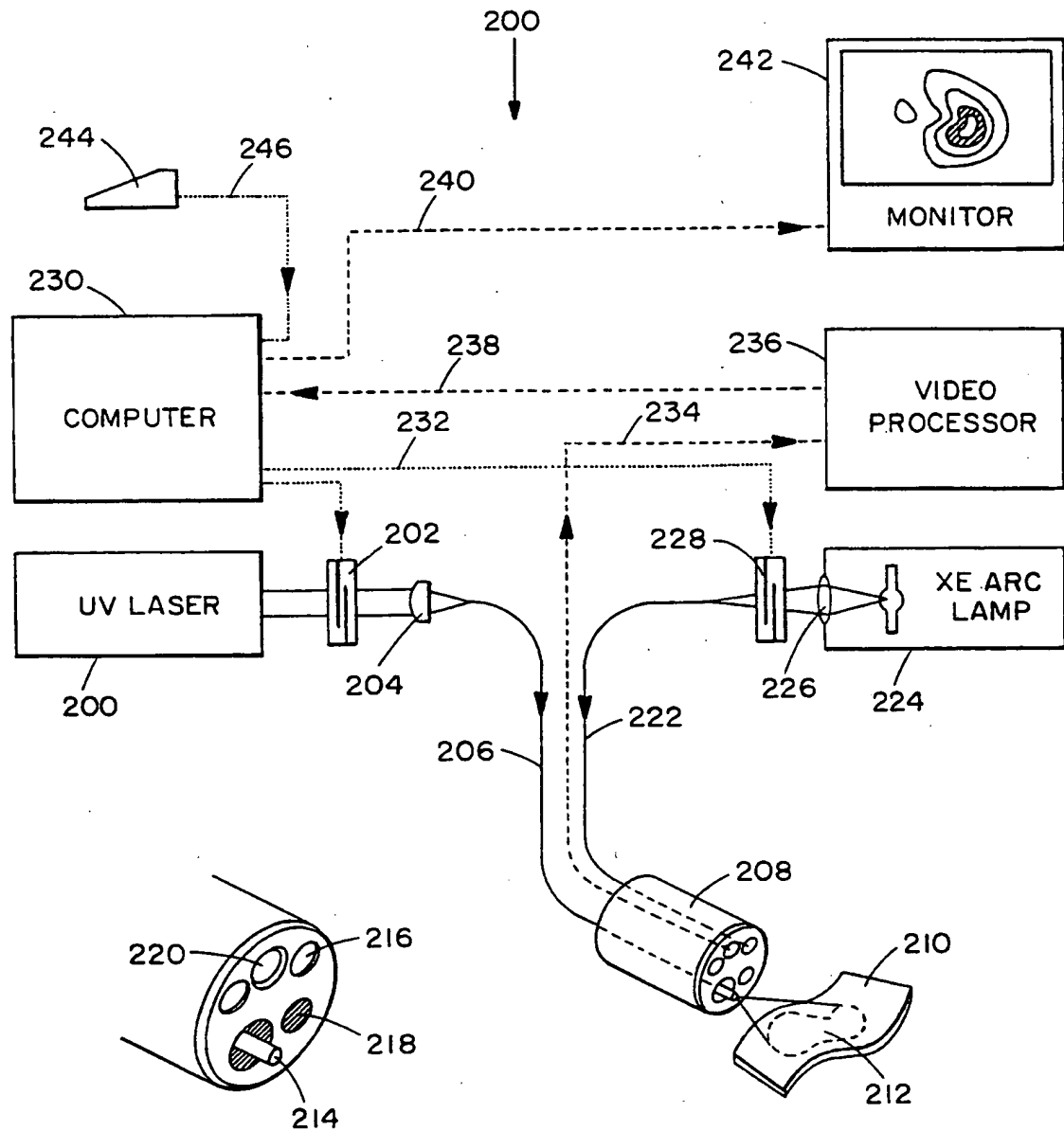


FIG. 13

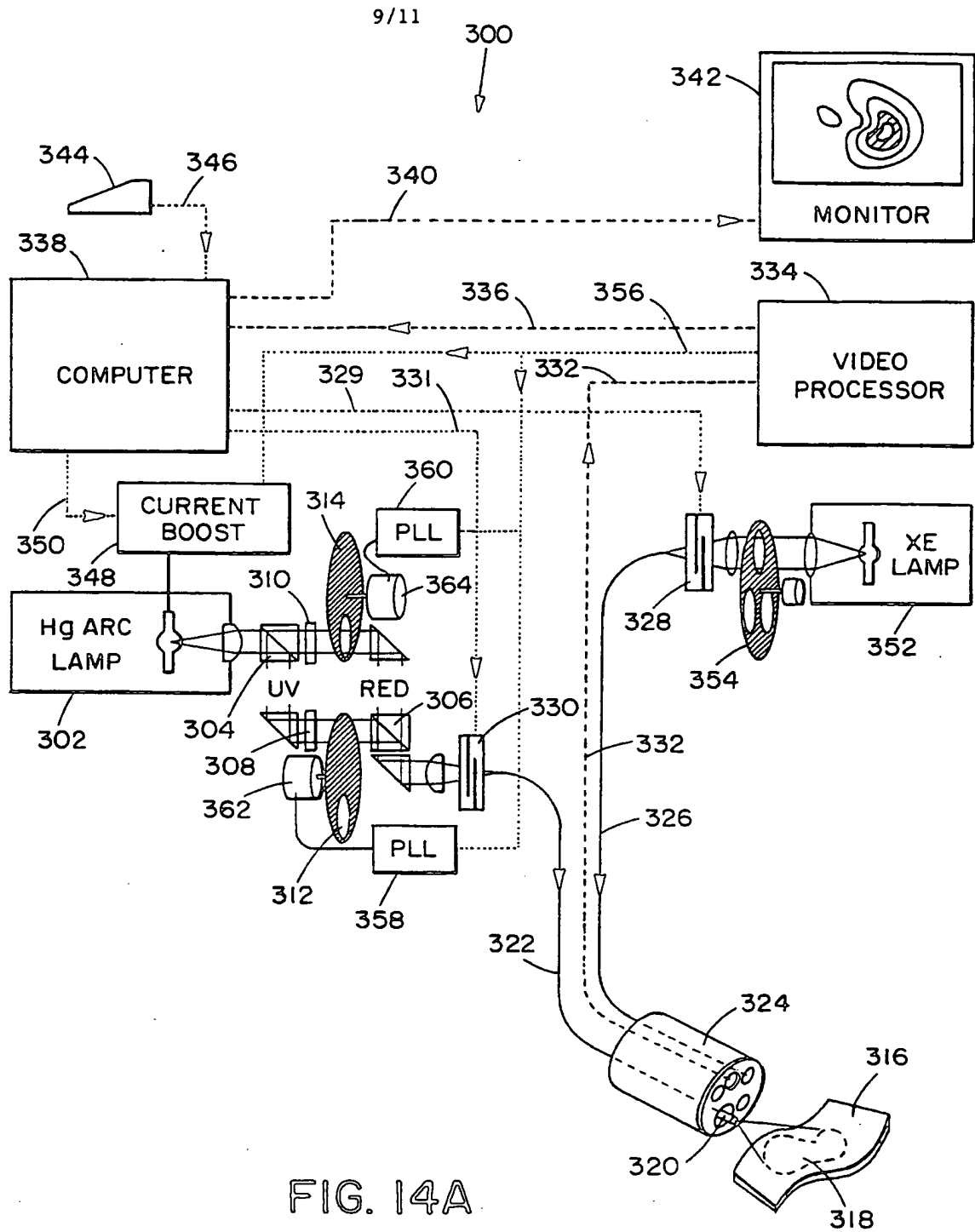


FIG. 14A

10/11

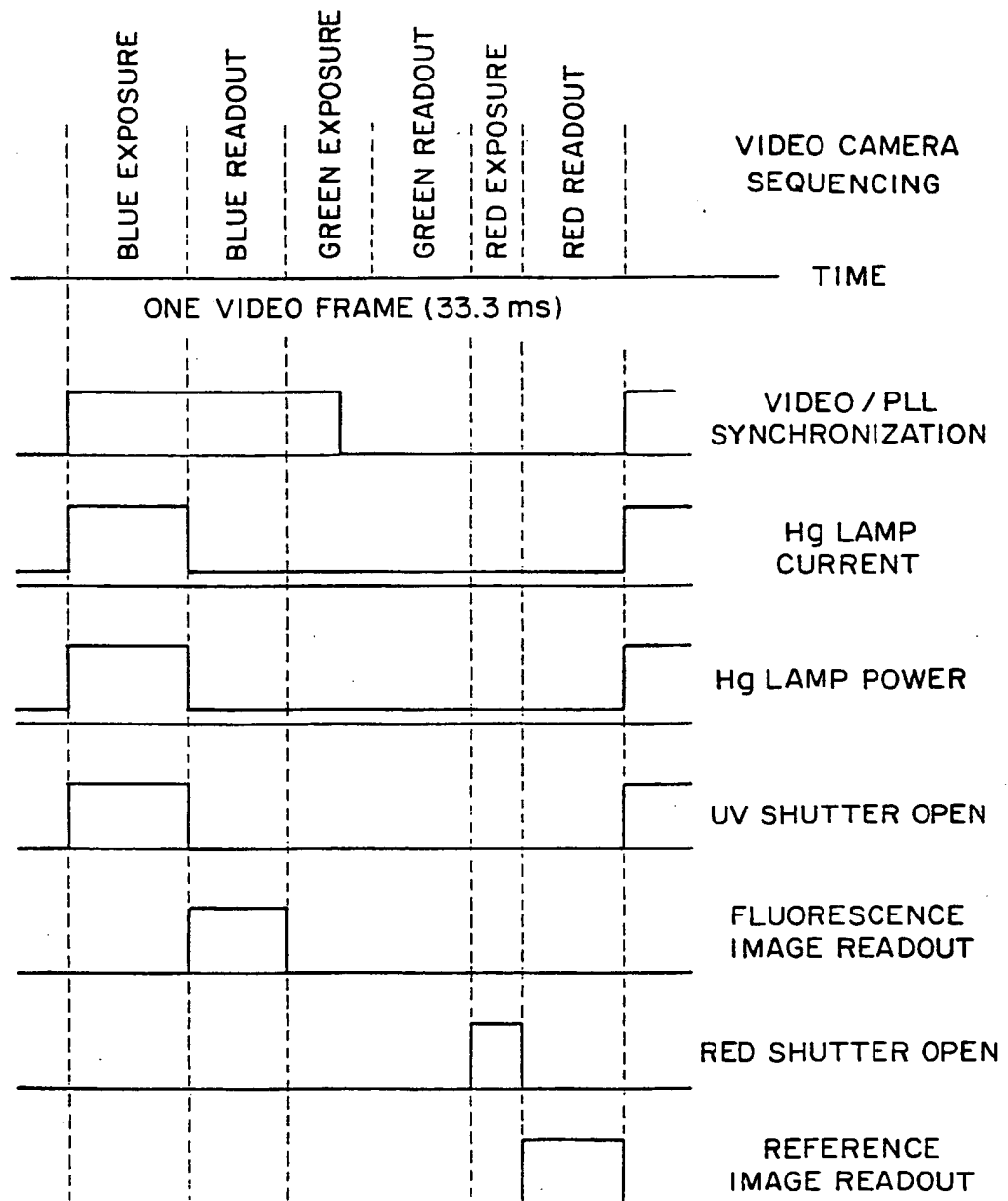
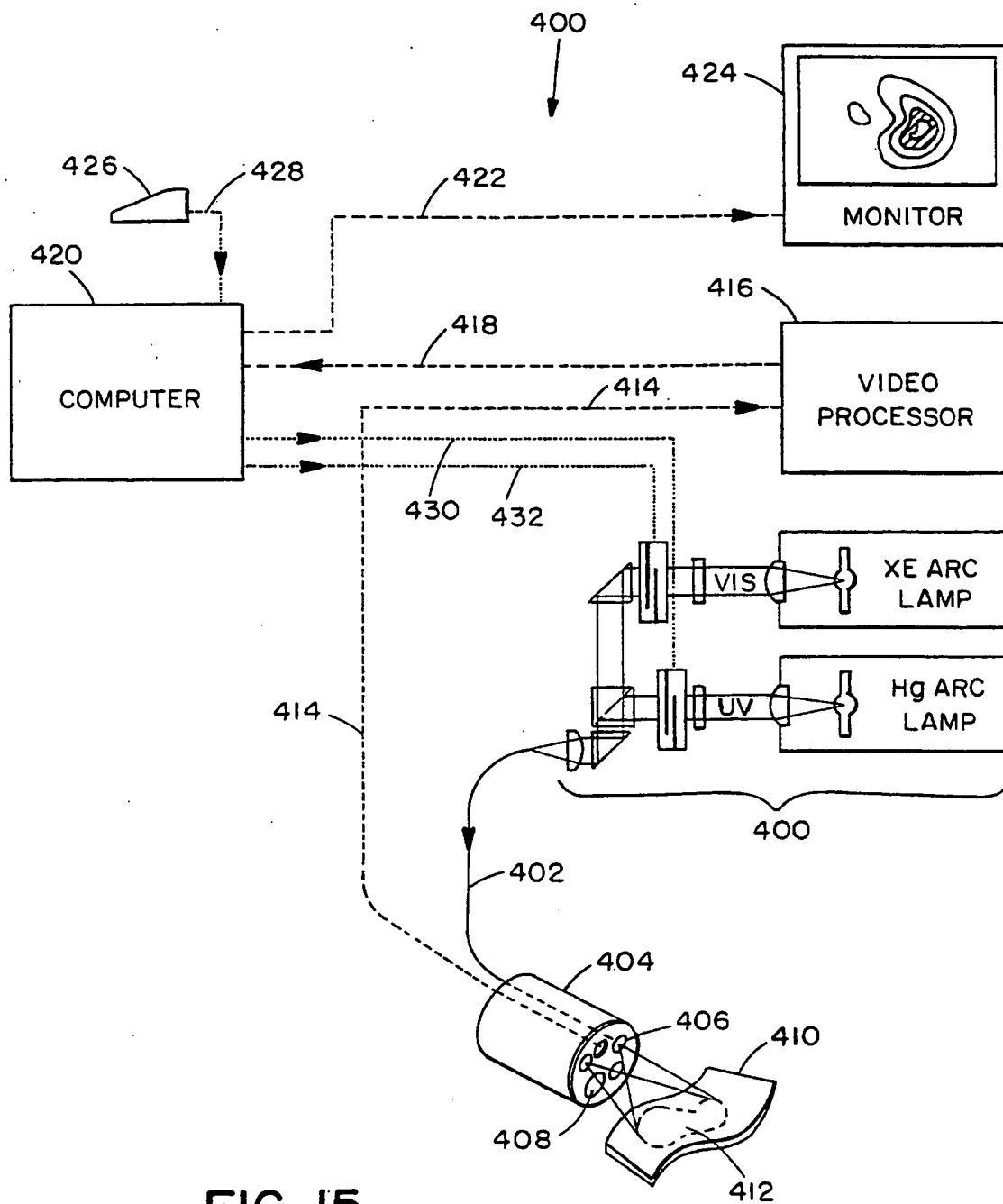


FIG. 14C

11/11

**FIG. 15**

INTERNATIONAL SEARCH REPORT

In ternational Application No
PCT/US 99/01723

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61B5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|-----------------------|
| X | US 5 187 572 A (NAKAMURA KAZUNARI ET AL) 16 February 1993 | 1,2,5,6, 9,12,13 |
| X | see column 3, line 67 - column 4, line 31 | 16 |
| A | see column 5, line 30 - line 47 | 3,4,7 |
| A | see column 11, line 43 - column 12, line 42 | 8,10,11, 14,15 |
| A | see column 15, line 37 - column 16, line 16 | 17-20 |
| | see column 19, line 61 - column 20, line 20 | |
| | see figures 1,11,28 | |
| X | DE 195 35 114 A (ASAHI OPTICAL CO LTD) 28 March 1996 | 12,15, 16,19 |
| A | see column 3, line 38 - column 4, line 3 | 1,2,5-7 |
| A | see column 5, line 14 - line 57 | 11,13 |
| | --- -/-- | |



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

17 May 1999

Date of mailing of the international search report

25/05/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Martelli, L

INTERNATIONAL SEARCH REPORT

In International Application No
PCT/US 99/01723

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication where appropriate, of the relevant passages | Relevant to claim No. |
|----------|--|--------------------------|
| X | WO 92 19148 A (ADAIR EDWIN LLOYD) 12 November 1992 | 12,15 |
| A | see page 3, line 18 - page 5, line 3 | 1,2,5-7 |
| A | see page 8, line 32 - page 11, line 17 | 11,13, 14,16 |
| --- | | |
| A | US 5 452 723 A (WU JUN ET AL) 26 September 1995 cited in the application see column 3, line 23 - line 32 see column 4, line 45 - column 5, line 14 see column 6, line 8 - line 15 see figure 1 | 1,5,6, 9-13, 15-17 |
| ----- | | |

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 99/01723

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---------------------|--|--|
| US 5187572 A | 16-02-1993 | JP 5084218 A DE 4136034 A | 06-04-1993 07-05-1993 |
| DE 19535114 A | 28-03-1996 | JP 8140928 A JP 9070384 A JP 9066023 A JP 8140929 A | 04-06-1996 18-03-1997 11-03-1997 04-06-1996 |
| WO 9219148 A | 12-11-1992 | US 5143054 A CA 2065976 A DE 69217216 D DE 69217216 T EP 0585321 A JP 6506855 T US 5251613 A | 01-09-1992 07-11-1992 13-03-1997 31-07-1997 09-03-1994 04-08-1994 12-10-1993 |
| US 5452723 A | 26-09-1995 | NONE | |